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On the Variations of Certain Factors in the Culture Ponds of Gold Fish, *Carassius auratus* L., in Special Reference to the Effect of Aeration into the Water*

(*With 3 Tables and 2 Figures*)

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Preface

The variations of certain factors in the culture ponds of gold fish, *Carassius auratus* L., have been studied at the Uota's Farm in Koriyama City, Japan.

The ponds in this farm, all contain stagnant water are grouped into three kinds in respect of their construction and utility.

Group A are ordinarily mud bottoms, surrounded by concrete walls without any artificial aeration in the water, group B are of a same construction except being supplied air babbles into the water by an air pump with 1/4 HP electric motor and group C are of concrete completely down to bottoms.

The writer has compared the changes of several factors in these ponds as follows: oxygen, carbon dioxide, temperatures, hydrogen ion concentrations in the pond waters, day and night. Especially, on the efficiencies of aeration into the water studies have been carried out.

Conditions of ponds employed and methods used

Of these three kinds of ponds above mentioned, the sizes and conditions have been investigated:

The Pond A employed, is 25 tubo (1 tubo is about two meters square) in area, almost of square shape and 60 cm in water depth and the transparency of this pond water was 2.5 cm by white color porcelain plate of 5 cm in diameter which was made by Dr. N. Nakamura (1954), for the abundant suspension of green micro-algae in the water.

By the observation of Mr. K. Akatsuka, the green colored water proved to contain mainly *Microcystis aeruginosum*, *Asplanchna producta*, *Cenedesmus*, *Crollera*, etc. with many other kinds of *Infusoria*. In this pond, young gold fish about 3 cm in body length, Ryukin, Wakin, Comett, etc. are reared about 1,000 in number.

*Aided by a grant from the Scientific Research Expenditure of the Department of Education.

The Pond B selected for the study, is situated by the side of Pond A, is 35 tubo in area, somewhat rectangle in shape and 50 cm in water depth and its transparency was 3.0 cm under the same conditions as that of Pond A; in this pond young gold fish such as Carico, Ranchyu, Ryukin, etc. of almost the same body length are reared about 1,500 in number, and in the center and four corners of this pond, unglazed pottery tubes are arranged, connected with rubber tubes to air compressor pump, to display small air babbles into the water all day long to avoid asphyxiation of those young gold fish.

The Pond C is 1 tubo in area, 20 cm deep and such a type of pond is usually employed for hatching eggs or for rearing fry of gold fish. Two ponds of this type were selected for our studies. In the one, named C-1, about 1,000 fry of Ryukin were rearing, fed *Daphnia morsei*, *Chydorus sp*, *Cyclops sp*, and no phytoplankton was observed but some green algae were seen on the surface of the concrete walls of the pond.

In the other one, named C-2, 12 fish, Oranda, Ranchu etc., body length from 6 to 10 cm were reared and the bottom of this pond was clearly observed by the transparency of the water in spite of some plankton of green micro-algae existing.

To study O_2 and CO_2 gas content in the water, portable VAN SLYKE gas apparatus was used and WINKLER's method was also employed for oxygen determination in the water about 5 cm under the surface. Merck Universal indicator was used to examine *pH* in the water.

Results of experiments

Table 1 shows the results of determination of O_2 and CO_2 in the waters of A and B Ponds and Table 2 also shows their results in the waters C-1 and C-2 Ponds, and the values of *pH*, changes in temperatures of pond waters and of atmosphere are shown in Table 3.

Table 1. Variations of gas contents in Ponds A and B.

Time	A Pond cc/*l*		B Pond cc/*l*	
	CO_2	O_2	CO_2	O_2
1 a.m.	—	—	28.8	1.9
3	—	—	31.4	1.3
4	31.4	0.0	—	—
6	—	—	33.8	0.6
6.30	31.1	0.0	—	—
8	—	—	38.1	0.0
10	—	—	26.3	3.9
11	19.1	8.7	—	—
13.30 p.m.	—	—	23.6	4.3
14	8.5	8.0	—	—

Table 2. Variations of O_2 contents in Ponds C-1 and C-2.

Time	C-1 Pond cc/*l*	C-2 Pond cc/*l*
2 p.m.	6.6	6.2
4	6.7	5.9
6	5.7	2.2
8	5.5	1.5
10	5.5	0.9
12	4.9	0.7
2 a.m.	3.4	0.5
4	2.9	0.5
6	2.9	0.7
8	4.7	3.5
10	5.2	6.2
12	6.6	7.0
2 p.m.	6.6	7.1

Table 3. The variations of temperature and *pH*.

	Time	1	3	5	7	9	11	1	3
X-22, 23, 1954	Air temp.	29.5	28.1	27.1	22.5	21.3	20.5	19.9	20.3
	Water temp. A	28.5	29.5	28.5	27.0	26.2	25.5	24.9	24.5
	〃 〃 B	26.2	26.7	26.8	26.2	25.8	25.4	25.0	24.6
	pH A	9.7	9.8	10.0	9.7	8.9	8.3	7.3	7.3
	〃 B	8.8	8.9	8.5	7.5	7.1	6.7	6.7	6.7
V-7, 8, 1955	Air temp.	—	19.9	19.6	18.7	17.4	15.7	14.9	14.4
	W. temp. C-1	—	20.1	20.0	19.5	18.6	17.5	16.6	16.4
	〃 〃 C-2	—	20.3	20.1	19.6	18.6	17.0	16.1	15.7

	Time	5	7	9	11	1	2.30
X-22, 23, 1954	Air temp.	19.2	24.2	25.5	27.1	28.1	28.5
	Water temp. A	24.1	23.7	25.4	27.7	29.5	29.5
	〃 〃 B	24.2	24.1	25.1	25.7	26.9	27.3
	pH A	7.3	7.5	9.8	9.8	9.8	9.8
	〃 B	6.7	6.9	8.1	8.5	9.7	8.5
V-7, 8, 1955	Air temp.	15.0	17.5	20.4	21.1	21.1	—
	W. temp. C-1	16.1	16.1	17.2	19.2	20.3	—
	〃 〃 C-2	15.5	16.2	18.0	20.0	20.7	—

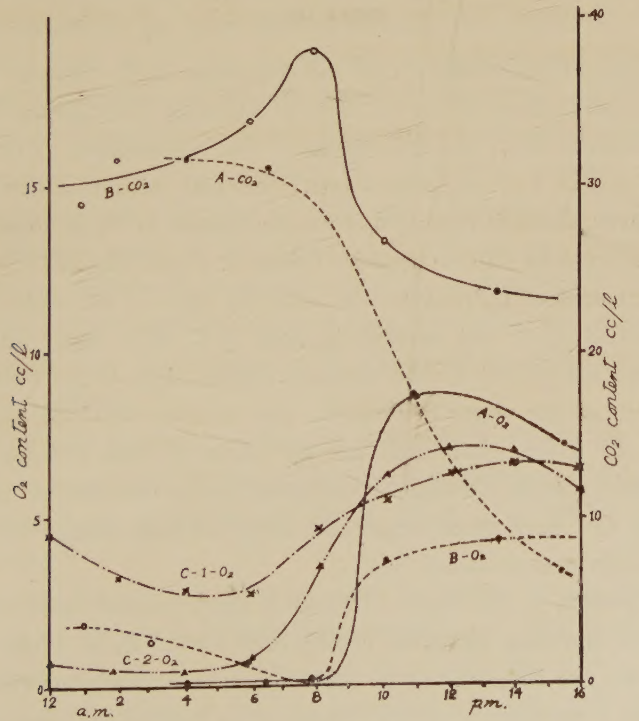

Figure 1. The variations of O_2 and CO_2 contents in the waters of the goldfish farms, with hours.

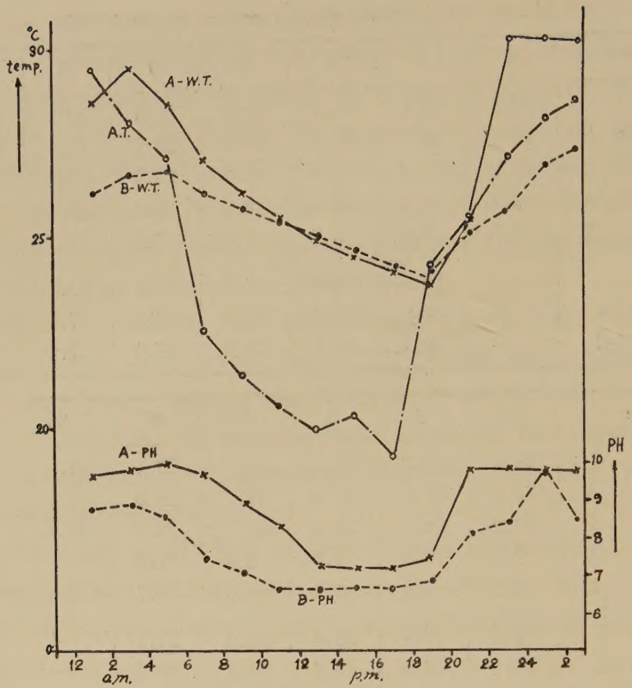

Figure 2. The variations of *pH* and temperatures in the goldfish farms in course of time.

Discussion

It is a natural phenomenon that the depression of O_2 content in the water of the ordinary fish farm in the early hours of morning (KAWAMURA, 1921, SUZUKI, 1923 and WATANABE, 1953), and the results of this experiments of O_2 variation in the waters of A (O_2), C-1 (O_2) and C-2 (O_2) were also coincide with that of natural variation, however, the different rates of the depression of O_2 in these three kinds of ponds were found as will be seen in Tables 1, 2 and Fig. 1.

The decrement of O_2 content was seen the most in the water of Pond A, the least in Pond C-1, and medium in Pond C-2. It is easily understood that these differences are caused by the amount of green algae in the water. Because, the transparency of the water in Pond A was 2.5 cm with the existence of a great amount of green planktons, but the water of Pond C-2 was transparent down to the very bottom, thought it contained some amount of plankton, and while in Pond C-1 such green organisms were not seen except the existence of some algae on the green colored wall.

The O_2 content in the water of Pond A down to zero from 4 to 8 a.m. in my experiments and then increased rapidly to 8.7 cc of O_2 in 1 liter water at 11 p.m.; these facts are supposed to be caused mainly by the phenomena of carbon assimilation and respiration of green planktons as well as the respiration of gold fish during day and night.

However, the changing course of O_2 content in the water of Pond B was much different from those of the other ponds mentioned above; the O_2 content in this pond gradually decreasing in the early hours of morning, reached zero only at 8 a.m. and the increment of it, being considerable was not so high as that of Pond A.

It is observed that these differences ought to be caused by the aeration in the Pond B, and by such mechanism Pond B was influenced to prevent the decreasing O_2 from reducing to zero in the early hours of morning until 8a.m., but it was not clear why the O_2 in the day time did not so much increase as that of Pond A.

The variations of CO_2 in Ponds A and B were also different and CO_2 in the Pond A decreased gradually from morning to afternoon with the course of time as shown in Figure 1, while that of Pond B did not show so much decrease as that of Pond A, but even showed an increase a while about 8 a.m.

From these results of variations of O_2 and CO_2 in the waters of Ponds A and B, it may be avered that aeration in the pond will not always be necessary for culturing fish in such a conditioned pond as mentioned above, and concluded that there could hardly be recognized the effective existence of aeration in this mechanism.

This is why: the asphyxiational symptoms called "Hana age" (to inspire air from the surface of the water) of all the gold fish during in the early hours of morning in Pond B as well as in Pond A appeared and the changes of O_2 increment and CO_2 decrement were observed in the water of Pond B, not so active as those in Pond A even under the aeration work with a compressor pump.

In Figure 2, there it shows about 6°C. difference between maximum and minimum temperatures from day to night and *pH* decreases in the early hours of morning, mainly caused by the increment of CO_2 in the water. Oxygen requirement for the respiration decreases when the temperature is depressed about 6°C. below the natural condition (Gardner and King 1922) and decreased value of *pH* may influence over the decrease of O_2 affinity of the blood of gold fish by the theory of Bohr's effects.

Then the gold fish had to show the symptom of "Hana age" by the results of asphyxiation on account of the extraordinary depression of the oxygen content in the waters of Ponds A and B and also of Ponds C-1 and C-2 in the early hours of morning.

But the gold fish did not die even when O_2 content of the water reduced to zero by the aid of the air inspiration with "Hana age", and it is also considered that such a strong resistance of living gold fish under the short run of O_2 may be caused by the low value of "*tu*" as Krogh and Leitch (1918–1919) had stated with the carp.

Summary

1. Studies on the variations of certain factors in the culture ponds of gold fish a special reference to the effect of aeration into the water have been carried out.

2. The variations of O_2 and CO_2 in the pond waters of gold fish coincide with those of an ordinary fish farm.

3. Those variations were caused under the influence of carbon-assimilation and respiration of fish and algae in the ponds.

4. The differences in the rates of variations are due to the various factors in the pond waters.

5. No good effect of aeration into the pond water was proved so far in this aeration method; all the fish studied here having showed a similar symptom of asphyxiation.

Acknowledgments

The author must expresses his hearty thanks to Mr. M. UOTA, the owner of the Uota's Gold Fish Farm and also expresses thanks to Messrs. R. INO and Y. KUSUKI who assisted me during the course of this experiment.

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Diurnal Rhythm in Phototaxis of Fish*

(*With 6 Tables and 7 Figures*)

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A study of the diurnal rhythm in the activities of animals, a field of biological investigation which is relatively new, should yield many fundamental facts of the physiology of the organism with respect to rhythmicity. It would be interesting to see what response to light at various times of day may be exhibited by some fishes.

Observation

To observe the rhythmicity in phototaxis as well as the response of fish to light, the same material must be used. However, due to the schooling effect on the fish by repeated trials under experimental conditions, different fish for each trial were used in the following experiment. Ten fish were used for each trial, and were placed in the experimental tank thirty minutes before observations were started so they could adapt themselves to the dark and become acquainted with their surroundings. The tank, which was located in a dark room, measured 95 cm in length, 45 cm in width and 30 cm in depth, the inside painted with lusterless black paint.

After turning on the light, the number of fish gathered within the illuminated field was recorded every thirty seconds and this counting was continued for fifteen minutes. In calculating, the mean of the figures recorded was converted into percentage and the term "gathering rate" was proposed for these values.

Using one white light, observation were made of the changes caused by the difference in the time of the day in the phototaxis of *Girella punctata* GRAY (body length about 2 cm), taken in large numbers near the Institute. As the light source, an 8 V light bulb attached to a cylinder so that the bulb might throw light on the surface of the water within a radius of 10 cm in diameter, was suspended at 15 cm above the surface of the water. In this case the area in which the fish gathered was divided into two parts, one a bright area (area A), 220 Lux, was 10 cm in diameter at the surface of the water, the other, a dim area (area B) for which a luxmeter reading is not available.

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* Contribution from the Ichthyological Research Institute.

The results are shown Table 1 and Fig. 1. There appear to be obvious

Table 1. Changes caused by the difference in the time of the day in the gathering rates for one white light (*Girella punctata*)

Gathering rate \ Time of day	15	16	17	18	19	21	22	23	24	1	2
Gathering rate for area A	72.3	82.0	68.0	61.0	32.0	4.3	2.3	3.7	3.7	5.0	3.0
Gathering rate for area B	12.3	12.3	11.3	35.7	40.0	64.7	13.7	28.7	45.0	18.7	21.3

variations in response caused by the time of day; gathering rate for area A was high in the day-time gradually becoming lower toward evening, and conversely ncreased in area B as the time of day approached evening. From evening to

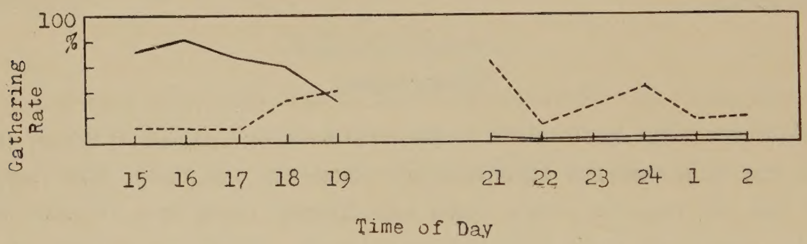

Fig. 1. Changes caused by the difference in the time of day in the phototaxis of *G. punctata*

——— area A
- - - - - area B

midnight large numbers of fish appeared in area B and, in the course of testing, the fish were relatively inactive and showed a tendency to keep away from the bright light.

The results of observations by the same procedure with two colored lights arranged at both sides of the tank are shown in Table 2 and Fig. 2.

Table 2. Changes caused by the difference in the time of the day in the gathering rates for colored light (*Girella punctata*)

Gathering rate \ Time of day	13	14	15	16	17	21	22	23	24	1	2
Gathering rate for green light	69.7	64.7	56.0	74.0	65.0	15.7	20.7	17.0	25.3	29.5	28.2
Gathering rate for red light	7.3	17.0	15.7	10.3	11.0	12.7	17.0	23.3	29.0	21.3	17.3

To obtain the colored light, Mazda-made color filters (V-G1, V-R2) were placed over the slits. The equipment was same as described in the previous paper by the authors.

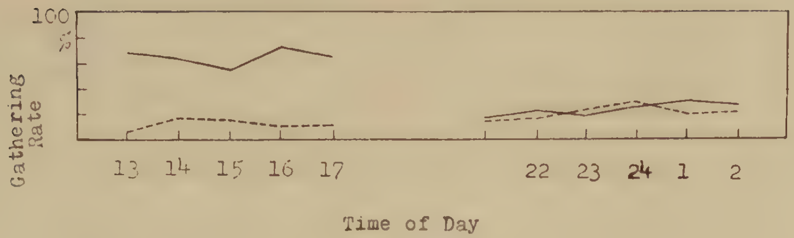

Fig. 2. Changes caused by the difference in the time of day in the phototaxis of *G. punctata*

——— Green light
- - - - - Red light

During the day-time, in view of the high spectral luminosity of the green light, it is natural that for the green light a higher gathering rate should appear than for the red light.

For the purpose of inquiring closely into the diurnal rhythm, observation were made throughout one day and the results are shown in Table 3 and Fig. 3. A notable break-down of phototaxis for green light is seen at a certain time of evening and simultaneously with it appears the extremely high gathering rate for red light, the third observation (**C** in Fig. 3) excepted. Such peaks of gathering rate in red light as this are seen at the middle of the night and also in the early hours of the morning while it is yet dark, in three of the four experiments. Throughout the above experiments, there was one obvious notable feature of this fish. It was found that they exhibited less activity during night-time than during day-time, avoiding the bright light or green light and usually remaining stationary at the non-illuminated bottom of the tub. With the approach of sunrise, the fish became active and were back to their day-time state. The graphs in Fig. 4 were prepared to express the change in aggregation of fish relating to duration of time and show that as the day approaches evening, the time required to show maximal aggregation gradually increased.

With regard to *Rudarius ercodes* Jordan et Fowler, the strong light-seeking tendency for green light was observed during day-time to be similar to that of *G. punctata*. Unlike the latter, however, when it gets dark the activity of *R. ercodes* decreases to a remarkable degree, and they no longer respond to light but fall asleep, sticking to the sides of the tub.

In general the fish called "nocturnal", for example, *Anguilla japonica* Temminck et Schlegel and *Plotosus anguillaris* (Lacépède), show no definite light-seeking tendency and they appear in larger numbers in the red light with spectral luminosity comparatively low, than in green light. An example of the results obtained by repeating the above experiments with *P. anguillaris* is shown in Table 4 and Fig. 5. No remarkable change of phototaxis was observed over a twenty-four hour period.

Table 3.

A

Gathering rate \ Time of day	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9
Gathering rate for green light	87.7	85.3	83.7	83.7	86.0	82.7	80.3	83.7	77.7	5.3	9.0	11.1	10.3	0.7	5.7	2.7	3.0	10.0	11.0	22.3	69.7	67.3	80.0	89.0
Gathering rate for red light	1.3	1.0	2.7	2.3	3.3	3.3	2.7	4.0	7.3	59.7	36.3	22.9	12.7	70.7	13.0	3.0	26.0	17.0	16.0	9.7	1.7	1.3	5.3	1.0

B

Gathering rate \ Time of day	16	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7
Gathering rate for green light	76.8	70.7	10.7	1.7	23.0	6.0	1.7	7.3	3.3	7.0	2.3	23.0	21.3	44.7	48.0	68.0
Gathering rate for red light	2.3	2.7	53.0	5.3	9.7	10.7	59.3	39.7	17.0	15.5	61.7	14.3	20.0	10.7	19.3	15.0

C

Gathering rate \ Time of day	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9
Gathering rate for green light	77.7	71.7	73.0	72.0	80.0	90.3	88.0	73.0	78.3	53.0	10.7	11.7	14.3	3.3	8.7	3.3	6.3	30.3	74.0	83.7	69.3	79.0	59.0	78.3
Gathering rate for red light	14.0	4.0	6.7	4.3	6.3	1.3	2.3	2.0	5.3	7.7	15.3	26.3	22.7	62.3	10.0	14.3	13.7	15.7	5.0	2.3	3.7	5.3	7.7	4.0

D

Gathering rate \ Time of day	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8
Gathering rate for green light	86.7	65.3	53.3	4.7	4.3	3.0	5.3	0	6.0	1.3	8.0	4.7	34.0	7.0	30.0	67.7
Gathering rate for red light	5.0	7.0	6.7	70.0	16.7	17.3	12.7	6.0	7.7	24.3	15.0	50.3	7.3	4.0	5.3	3.0

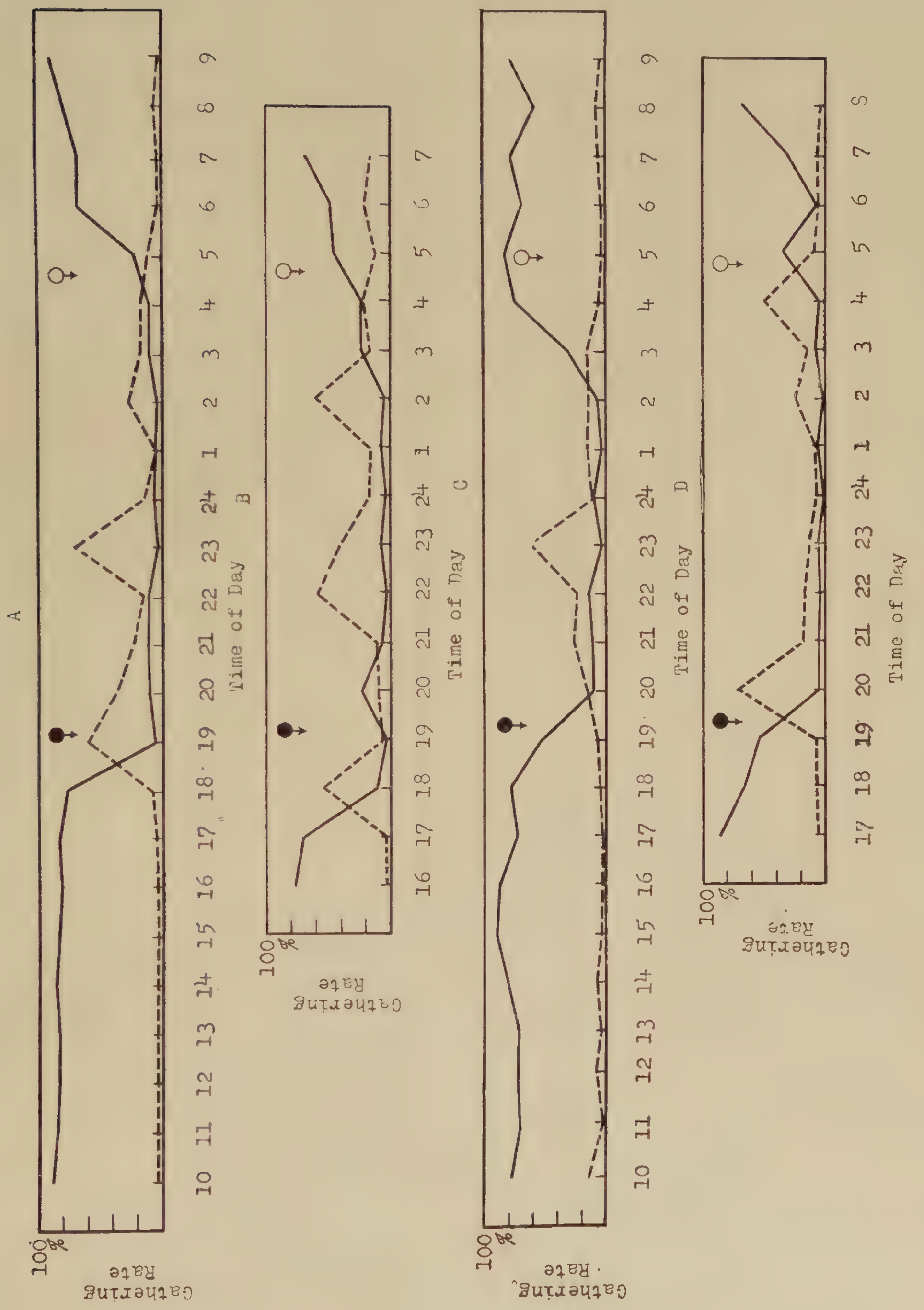

Fig. 3. Diurnal rhythm of the phototaxis in *G. punctata*

A: June 6, 1950, B: June 8, 1950,
C: June 18, 1950, D: June 30, 1950.
——— Green light ● sunset
- - - - - - Red light ♀ sunrise

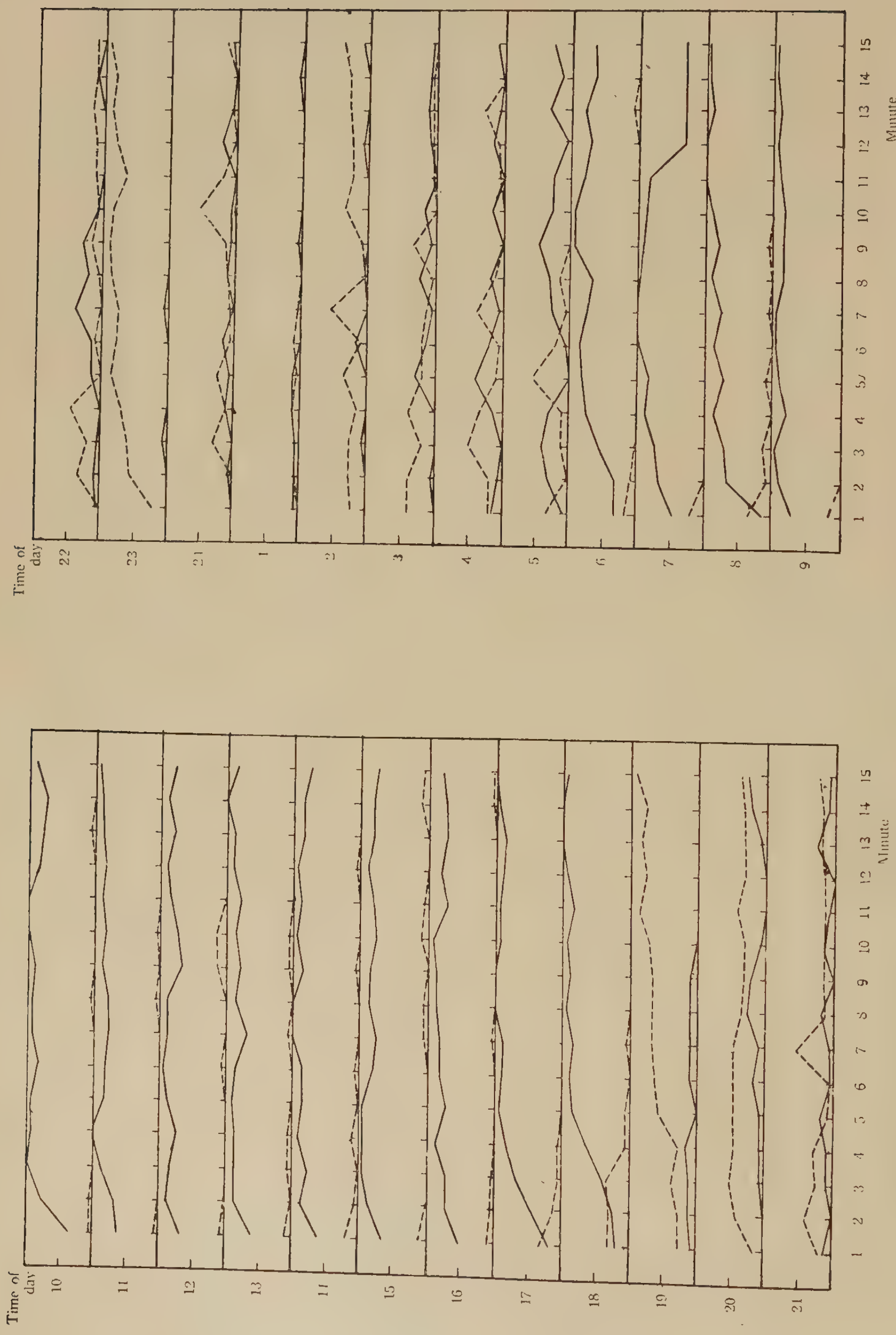

Fig. 4. An example of the change in aggregation of fish with regards to duration of time. Aggregating rate vertical.

Table 4. Changes caused by the difference in the time of the day in the gathering rates for colored light (*Plotosus anguillaris*)

Gathering rate \ Time of day	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7
Gathering rate for green light	18.7	36.7	13.3	11.3	20.0	8.7	15.3	6.0	20.7	10.0	12.0	12.7	10.0	5.3	1.3	6.7	16.0	17.3	16.0	10.0	14.0	14.7	13.3	11.3
Gathering rate for red light	14.0	18.0	49.3	16.0	24.7	18.7	20.7	32.7	37.3	30.0	27.3	18.7	20.0	20.0	6.7	33.3	24.0	23.3	8.0	34.7	16.7	12.0	15.3	35.3

Table 5. Changes caused by the difference in the time of the day in the gathering rates for colored light (*Mugil cephalus*)

Gathering rate \ Time of day	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1	2	3	4	5	6	7
Gathering rate for green light	36.7	42.3	27.3	34.7	27.0	33.3	38.0	33.0	40.3	43.7	43.0	32.2	36.7	35.3	32.7	33.7	24.7	43.3	34.7	27.0	30.0	41.0	35.3	33.0
Gathering rate for red light	6.7	3.7	5.0	6.7	6.0	6.7	8.0	7.3	12.3	12.0	12.7	20.0	11.0	14.0	18.0	16.7	14.0	13.7	9.7	14.0	19.3	14.0	15.3	18.3

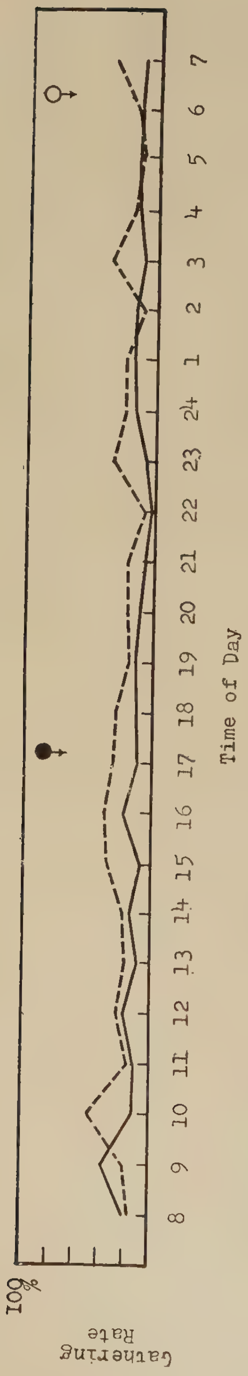

Fig. 5. An example of the diurnal rhythm of the phototaxis in *P. anguillaris*.

—— Green light ● Sunset
- - - Red light ○ Sunrise

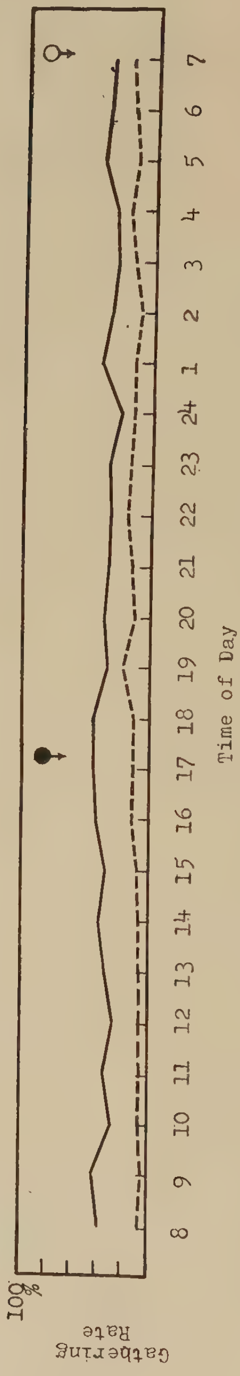

Fig. 6. An example of the diurnal rhythm of the phototaxis in *M. cephalus*.

—— Green light ● Sunset
- - - Red light ○ Sunrise

For the fish of other group, *Mugil cephalus* LINNE was used mainly in this test. As are shown in Table 5 and Fig. 6, there does not appear to be any remarkable variation in diurnal rhythms. The gathering rate in green light for most fish in this group is usually not more than about 40%.

In the course of all of the above experiments as far as practicable, careful attention was paid to the elimination of changes in environmental conditions, such as temperature (19.3–22°C), pH value and oxygen content of the water, which might have a pronounced effect upon the metabolic rate.

Discussion

In higher animals, so closely correlated is the mode of life of the animal with the structure of the retina that, from a histological section, one can predict something of the habits, as well as the visual ability of the animal.

With regard to fish, generally spoken of as bright-light fish or dim-light fish, the fundamental and interesting correlations between their habits and the detailed structure of visual cells and pigmented epitherium, have been clarified by many workers.

According to TAKATUZI, *G. punctata*, *R. ercodes* and *Halichoeres poecilopterus* (TEMMINCK et SCHLEGEL) and a few other fish possess a pure cone retina equipped for day vision. *H. poecilopterus*, were markedly inactive when it became dark and fell asleep on their sides out of sight under the shelter.

In view of the above facts, the authors first considered that it may well be allowed to discuss thoroughly the results of our experiments from a point of view of histological evidence of visual cell. It seems to the authors that we could expect the high threshold of the cones to have some bearing upon the remarkably high light-seeking tendency during day-time and inactivity at night. Furthermore, we speculated that the diurnal rhythms exhibited by *G. punctata* etc. are the outcome of acquiring a habit from the actual alternating of day-time light and night-time dark. And in the natural state, the fish gradually lose the power of sight as the light becomes more and more dim with decreasing light intensity and thus rhythmic alteration of light and dark brings a noticeable recurring diurnal rhythmicity which they retain even in the laboratory. However, we found one fact, that the retinae of *R. ercodes* possess a large number of rod cells (unfixed retina). We also found that their dark-adapted retina contains reddish photosensitive pigment of a property as yet unknown. It may be rhodopsin or porphyropsin. Considering these facts, it may not be sufficient to discuss the diurnal rhythmicity from only the one viewpoint of retinal structure. For the elucidation of these phenomenon, there remains much to be learned about the physiological mechanism of the diurnal rhythmicity of animal behavior. We can not give any explanation of the mechanism of the diurnal rhythmic

activity observed in our experiment. The diurnal rhythms occurring in the internal physiological conditions caused by the rhythmical changes of the environmental factors may bring about these diurnal rhythms in the activity of fish. It is interesting to note that such a remarkable rhythmicity in *G. punctata* as above mentioned, can not be found in fish that have been subjected to continual darkness for a week (Table 6 and Fig. 7). We need to have an explanation of the peaks of gathering rate which appeared for red light during night-time in Fig. 3.

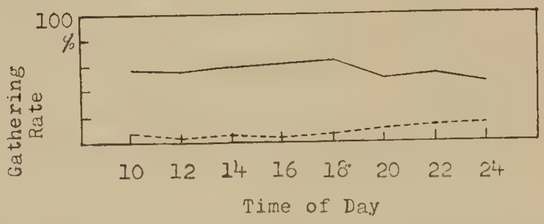

Fig. 7. Changes caused by the difference in the time of day in the phototaxis of G. punctata that have been subjected to continual darkness for a week.
——— Green light ------- Red light

Table 6. Changes caused by the difference in the time of the day in the gathering rates of G, punctata that have been subjected to continual darkness for a week.

Time of day / Gathering rate	10	12	14	16	18	20	22	24
Gathering rate for green light	58.3	56.3	59.7	62.3	64.7	50.7	52.7	46.3
Gathering rate for red light	7.3	2.0	6.3	3.3	7.0	11.0	12.0	15.3

Summary

1. These experiments have been made in order to observe diurnal rhythm, or responses to light at various times of the day which may be exhibited by some fish.

2. As compared with some fishes, certain fishes, for example *Girella punctata*, *Rudarius ercodes*, show an extremely strong light-seeking tendency in day-time and a notable break-down of activity in night-time.

Acknowledgment

In conclusion, the authors wish to express their sincere gratitude to Dr. K. MATSUBARA, Professor of Kyoto University, for their kind suggestions.

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Vol. 2, No. 1, Oct. 30, 1955.

The Observation of the Surface Structure of the Cultured Pearls Relating to the Color and the Luster[1)]

(With 3 Plates, 1 Text-figure and 6 Tables)

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Introduction

Cultured pearls[2)] are mostly composed of the step-like accumulation of scaly laminae about 0.5μ in thickness, consisting of conchiolin membrane and aragonite crystals deposited on the membrane. The margins of laminae are exposed to the surface and exhibit various striped patterns, caused by the modes of crystal growth of aragonite.

Generally, the colors of cultured pearls are considered to depend on their chemical constituents and laminary structures.

KOSAKI (1947) reported that amount of porphyrin bodies has a relationship with kinds of pearls. For instance, pink pearls contain relatively larger amount of porphyrin, to show beautiful fluorescence, and the metalloporphyrin consisting of Pb or Zn salts, which may exhibit red fluorescence. On the contrary, according to TANAKA (1955), the amount of porphyrin in pearls is so small that it gives no effects to the color, and new pigments exist in pearls.

TAKAOKA (1955) found that pink pearls contained relatively large amount of Mn, while its content in silver pearls was of a trace.

On the other hand, UCHIDA and UEDA (1947), and OMORI (1948) studied the color or reflection spots—surface colors—of cultured pearls, and found that they were due to the constructive interference of light reflected from the laminae of pearls. According to the former authors, the thickness of a lamina was 0.41μ in a pink pearl, and 0.55μ in green one. The present author (WATABE, 1950) also obtained the same results.

But, in comparison with many studies above mentioned, little have been done about the relationship between the surface structure and the color or the luster of the cultured pearl.

In the present paper, the surface structures of cultured pearls and the relations mentioned above are discussed.

The author expresses his sincere thanks to Dr. Yaichiro OKADA, Dean of this Faculty for his instructive directions and kind encouragements. Thanks

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1) Aided by the Scientific Research Fund from the Ministry of Education.
2) In this paper, the term "pearl" means only the nacreous layer pearl.

are also due to Dr. Keiichi OMORI, Professor of TOHOKU University for his useful advices. Electron micrographs were taken by the courtesy of Dr. Bunya TADANO, HITACHI Central Research Laboratory, whose kindness is greatly appreciated.

I. Surface structures of cultured pearls

The surface structures of cultured pearls can be divided into the following 4 types.

1) The surface with no striped pattern.

Two configurations are in this type.

a) The surface has a homogeneous structure, and no distinct pattern is discernible under the optical microscope (Figure 1).

The surface is considered to be formed as follows.

During the course of the cultivation of Japanese pearl oysters, *Pinctada martensii* (DUNKER), it happens that the nacre secretion is caused to cease by some reasons. That is, when they are hibernating in winter in the water below 13°C, or when they are landed to be cleaned off their shells from adhered small creatures and weeds, they often do not secret nacre substance. And, as reported before (WATABE, 1952), when the secretion begins again, a thick conchiolin membrane is formed to cover the whole surface of the pearl. If the pearl is removed just at that time, the surface shows a homogenous structure, because it is composed of this membrane only.

However, it is a very rare occasion to meet this case. Though the surface shows the homogeneous structure under the optical microscope, the electron microscopic observations often reveal that very fine aragonite crystals has already deposited on the membrane. These crystals are shown in Figure 3.

b) The surface composed chiefly of the aggregations of smaller aragonite crystals about 1μ in size, showing no striped patterns. Each crystal exhibits an irregular form and the mode of aggregation is irregular, too. Pearls provided with this surface constitution show white and opaque appearance. They are called " Shio-Dama ", and harvested more often in summer than in winter.

In most cases, the irregular aggregates do not cover entirely the underlying layers. There is no connection between the aggregates (A in Text-figure 1) and underlying layers (B).

The present author reported the formation of the surface constitution (WATABE, 1951). In brief, when pearl oysters are active in summer, $CaCO_3$ solutions are secreted on the surface of pearls in oysters, and so when they are taken out of the oysters, crystallization may take place rapidly. This will cause the formation of opaque irregular aggregates of smaller aragonite crystals.

2) The surface with irregular pattern.

This surface is formed by the irregular accumulation of laminae (Figure 2).

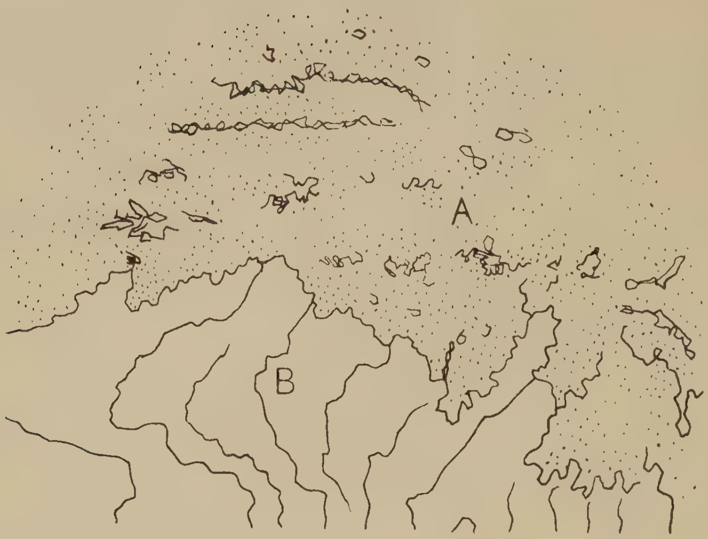

Figure 1. Showing the aggregations of smaller aragonite crystals (A) covering the normal laminae (B). There is no connection between (A) and (B).

3) The surface with parallel striped pattern (Figures 4 and 5).

This surface usually consists of typical step-like accumulation of laminae, and edges of laminae run in parallel one another.

As seen in Figure 4, a surface has isolated crystals along the edges of the laminae. This fact, or detailed observations of the crystal arragement (WATABE, unpublished) indicate the direction of the horizontal growth of a lamina running at right angles to the stripe. Therefore, the parallelism of the stripes means that these laminae grew in the same direction one another. Moreover, it is conceivable that where the laminae grew at the same rate, the stripes should come to be linear.

4) The surface with volute pattern.

Two kinds of pattern are distinguished in this type. The one indicates the concentric step-pattern just like the contour-lines of a map (Figure 6), and the other, the spiral steps (Figure 7). In both cases, the central parts are higher than the marginal parts. Directions of the crystal growth run radially from the center of the spirals. (The direction in these cases is different from that of the surface in Figure 12.)

Recently, growth spirals in crystals have been discussed by the dislocation theory, and the mechanism of the formation of spiral pattern in pearls can be explained by the modification of this theory (WATABE, unpublished).

II. Detailed description of the structure of laminae

Though the rough classification of surface patterns are as above mentioned,

detailed observations show that the mode of aggregation of crystals which constitute the laminae is varied, even if their surfaces show the same type of pattern.

As described before, the direction of horizontal growth of aragonite crystals runs at right angles to stripes, so isolated crystals are scattered along the edges of laminae at an earlier stage of crystallization (Figure 8). In some cases, these little crystals deposit even on the surface of the inner part of the laminae (Figure 9). During the growth, these crystals grow up larger and larger, or smaller crystals are swallowed up by larger ones, and finally, crystals come to combine together. These aggregates develop either in parallel or irregular growth of crystals.

Now, when the 001 planes, that is, the horizontal surfaces, are flat, crystals are brought together so well compacted that the outlines of crystals can hardly be discernible in the inner parts of the laminae, some holes being left near the marginal parts. (The number of holes seems to be less in the case of irregular growth than the parallel one.) Thus the surface of the lamina comes to be flat (Figure 10). But, as shown in Figure 11, when 001 planes take curved surfaces, the outlines of combined crystals can clearly be discernible, and the surface of the lamina is quite uneven. The results of the observations so far tell that these 001 planes appear only in crystals which developed in irregular growth.

There are some types of the surface to be unconsistent with the description, "pearls are formed by the step-like accumulation of scaly laminae".

In one stage of the pearl formation, its surface is composed of crystal aggregates with the same level. The surfaces mentioned in §1, b) belong to this example. Another example is found in pearls in a little more advanced stage of crystal growth. Though these pearls have irregular, parallel, or volute patterns on the surfaces, their laminae do not form the steps; they are made of aggregates of dendrites on the same level.

The process of the formation of these configurations is considered as follows. First, crystals deposit here and there on the thick conchilolin membrane which cover the whole surface of a pearl (see § I, b) at an earlier stage of a pearl formation. When they develop in dendritic growth, the neighbouring main stems will run parallel one another in some condition of the $CaCO_3$ solutions, or in some cases, main stems will gradually bend to form the volute pattern (Figure 12). In other cases, dendrite will develop spontaneously. In all cases, crystals branched from each of neighbouring two stems come to contact together, and a single sheet of crystal aggregate is formed. Figure 12 indicates this configuration (cf. Figure 7). The direction of horizontal growth in these cases runs parallel to the stripe (WATABE, 1954).

Theoritical considerations about the formation of parallel or volute pattern in these cases will be reported in detail in another paper.

If the laminar growth advances by successive deposition of crystals, step-like patterns will be formed at last.

III. Relations between the surface structures and the color or luster of cultured pearls

1) Color

In the nacreous layer of shells of molluscs, all three types of surface pattern, irregular, parallel, and volute, are discernible in one shell, though there is a tendency that parallel pattern is formed along the margin, and volute in the inner part of a shell (SCHMIDT, 1923). But, generally, one pearl indicates one type of pattern. Moreover, the distance of the stripes is in the order of 10 or 20μ. So, BREVSTER (1841) thought that colors of pearls were due to the interference of light by those echelon gratings. UMEKAWA (1928) reported that the distance of stripes was larger in pink (22μ in average), or golden (22μ) pearls than in green ones (18μ). But, SCHMIDT (1923) had an opposite opinion. He reported those echelon gratings took only little part in colors of pearls.

So, the present author examined the stripe distance, and surface patterns of pearls, pink, yellow, and green. (Pearls with other colors were excluded as their number was so scarce.)

Table 1 shows the average value of the distance of stripes in each color. The estimation was made with the confidence coefficient of 95%. No difference was found in the distances of the stripes to the color.

Table 1. Mean value of the distance of surface stripes of pearls.

Color	Mean distance (in μ)	Fiducial limits (Confidence coefficient 95%)
Pink	24.03	±1.35
Yellow	25.33	±3.61
Green	20.96	±2.49

Table 2. Contigency table for the surface pattern and color of good lustered pearls.

Color \ Surface pattern	Irregular	Parallel	Volute	Total
Pink	9	15	13	37
Yellow	8	17	11	36
Green	9	14	9	32
Total	26	46	33	105

Secondly, number of pearls with irregular, or parallel, or volute patterns were listed on Tables 2 and 3. For convenience, pearls with no striped patterns were included in the column of irregular pattern. Examination of Tables 2 and 3 showed that the rate of appearance of each surface pattern in each color was

Table 3. Contigency table for the surface pattern and color of bad lustered pearls.

Color \ Surface pattern	Irregular	Parallel	Volute	Total
Pink	2	13	11	26
Yellow	1	8	5	14
Green	1	7	4	12
Total	4	28	20	52

equal with the significance level of 95%.

Those results showed that the surface pattern or the intervals of stripes had no relation to the color of pearls. This fact agrees well with SCHMIDT's opinion. If a pearl were not of a spherical form, but to take a platy one like a shell, the interference effect of light by echelon gratings on the surface would appear. But, actually, its surface is curved, so the effect will be diminished after all.

2) Luster

It can be easily understood that a luster of a body depends on its surface structure, and an even surface gives a splendant luster, while uneven surface gives a dull one.

From this view point, it was examined whether the luster of pearls would differ by the grade of smoothness of their surfaces or not, in other words, by the modes of crystal aggregation or surface patterns. Number of pearls with varied characteristics are listed in Tables 4, 5 and 6.

Then, the following results were obtained.

1) Pearls with homogeneous structure in § 1, b) always have dull luster. Irregular pattern is found more often in bad lustered pearls.

Table 4. Contigency table for the surface pattern and the luster of pearls.

Luster \ Surface pattern	Irregular	Parallel	Volute	Total
Good	4	28	20	52
Bad	26	46	33	105
Total	30	74	53	157

Table 5. Number of good and bad lustered pearls with aragonite crystals the outlines of which can clearly be seen in the surface laminae.

Luster	With clear outlines of crystals	Others	Total
Good	10	42	52
Bad	43	62	105
Total	53	104	157

Table 6. Number of good and bad lustered pearls with little aragonite crystals scattered on the surface laminae.

Luster	With little crystals on the surface laminae	Others	Total
Good	18	34	52
Bad	61	44	105
Total	79	78	157

2) The rate of appearance of bad lustered pearls is higher where the outlines of aragonite crystals with the curved 001 planes can clearly be seen in the surface laminae.

It is also higher where little crystals are scattered on the surface of the laminae.

In conclusion, it was found that the surface structures of cultured pearls have no relation to colors, but have a deep relation to the luster.

Summary

I) The surface structures of cultured pearls were studied with the help of optical and electron microscopes. They were classified into 4 types.

1) The surface with no striped pattern.

There are 2 types in this case, the one is composed of a thick conchiolin membrane which was formed at the first stage of nacre formation, the other composed of the aggregates of fine aragonite crystals which were formed by rapid crystallization in summer.

2) The surface with irregular pattern.

3) The surface with parallel pattern.

4) The surface with volute pattern.

The direction of horizontal growth of the lamina runs at right angles to the line on the surface.

At an earlier stage of nacre formation, aggregates of crystals deposite on the same level and do not form the steps, though they show parallel, volute, or irregular patterns. In this case, the direction of the line of the surface coincide with that of the horizontal growth of the aggregates.

Theoretical considerations about the formation of each pattern will be reported later in detail.

II) The surface structures of cultured pearls have no relation to the colors but have a deep relation to the luster.

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Explanation of Plates

Surface structures of cultured pearls.

Fig. 1. The surface with homogeneous structure, no distinct pattern is discernible under the optical microscope.

Fig. 2. Irregular pattern.

Fig. 3. Electron micrograph of the structure shown in Fig. 1. Little aragonite crystals have already deposited.

Fig. 4. Parallel pattern, isolated crystals scattered along the edges of the laminae.

Fig. 5. Parallel pattern, no isolated crystals along the edges.

Fig. 6. Volute pattern. Concentric steps.

Fig. 7. Volute pattern. Spiral steps.

Fig. 8. Electron micrograph of isolated crystals along the edges of the lamina.

Fig. 9. Electron micrograph of isolated crystals on the surface of the inner part of the lamina.

Fig. 10. Electron micrograph of the laminae with flat surfaces. Holes are left near the marginal parts.

Fig. 11. Electron micrograph of crystals with curved 001 planes. Their outlines are clearly be seen.

Fig. 12. Volute pattern without steps.

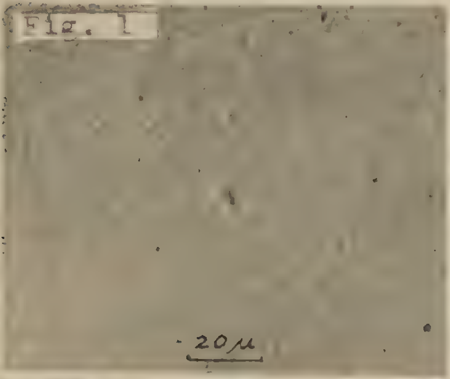


Fig. 1

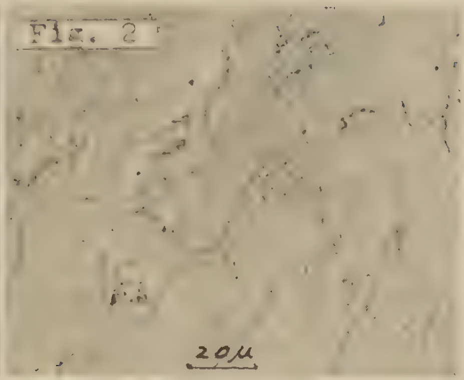


Fig. 2

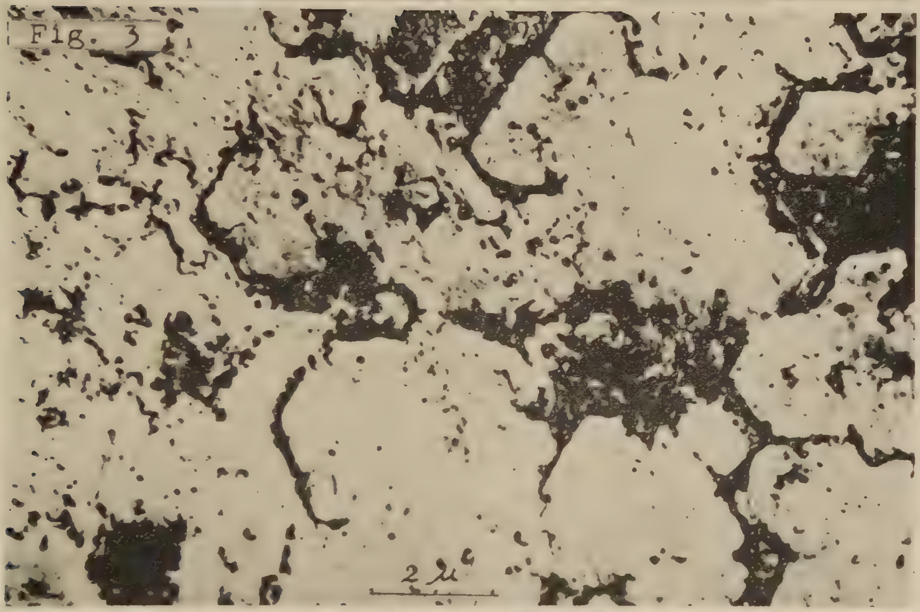


Fig. 3

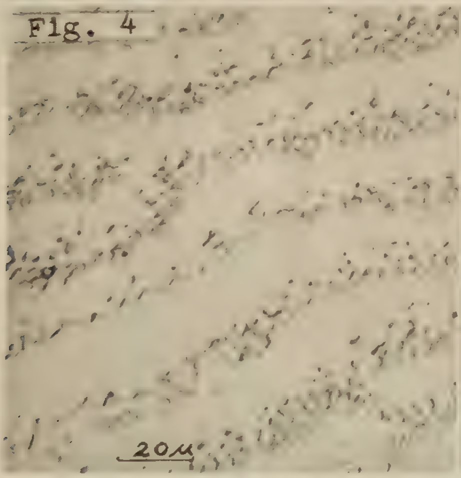


Fig. 4

Fig. 5

Fig. 6
20µ

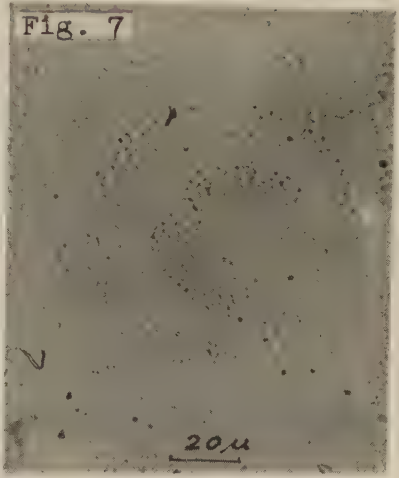


Fig. 7
20µ

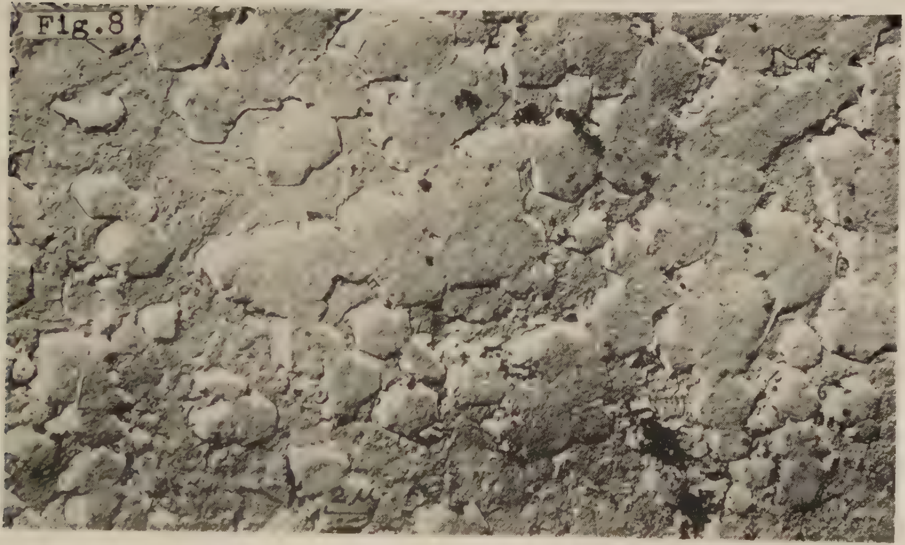
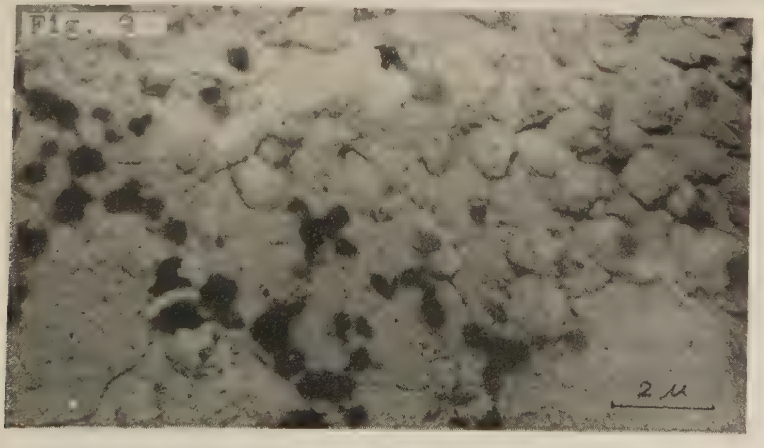


Fig.8


2µ

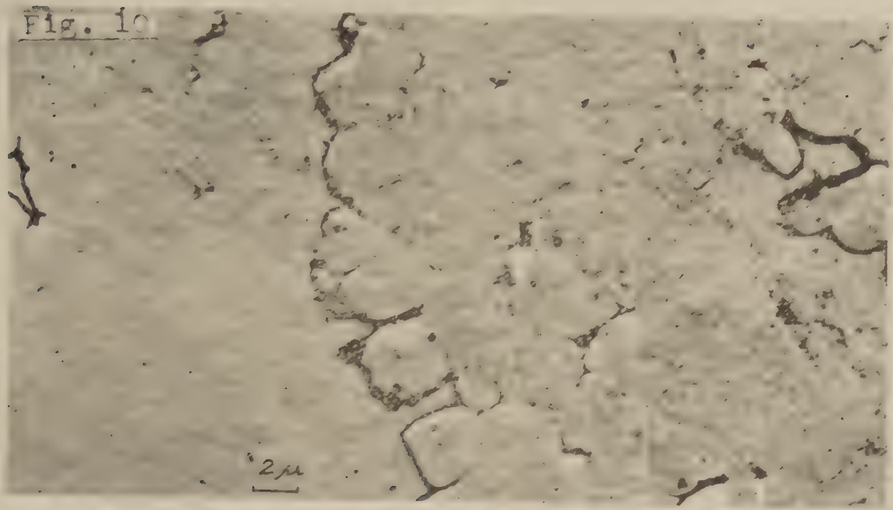


Fig. 10
2μ

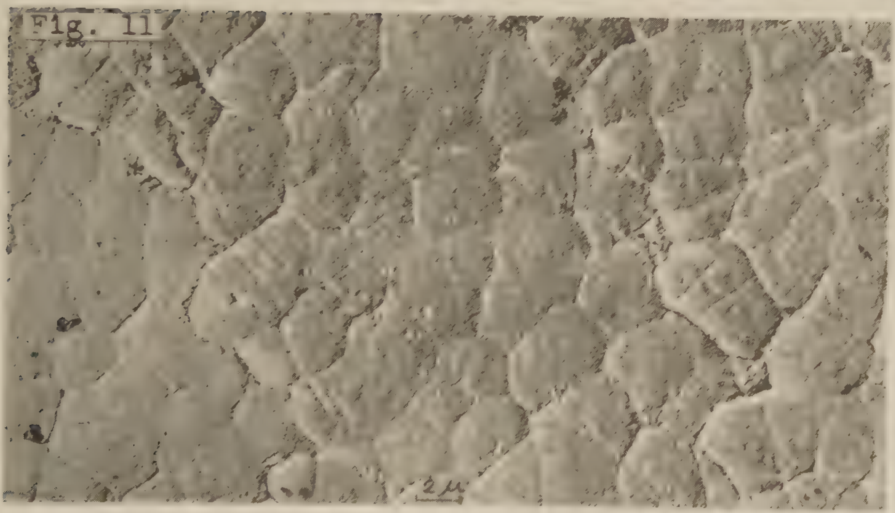


Fig. 11
2μ

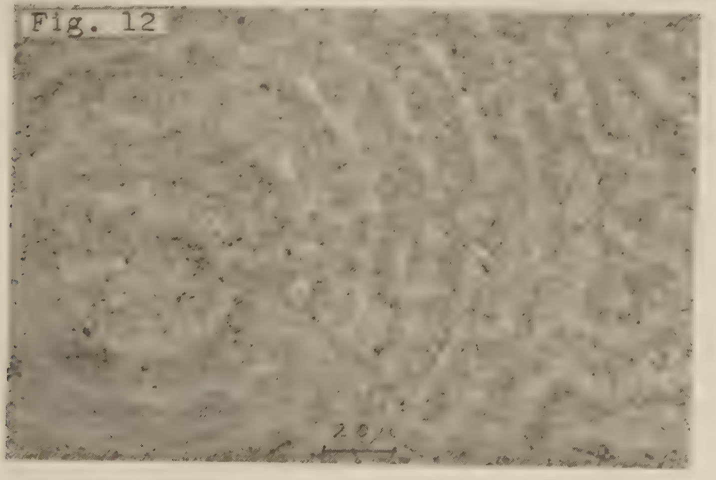


Fig. 12

Report of Faculty of Fisheries,
Prefectural University of Mie,
Vol. 2, No. 1, October 30, 1955.

Notes on Liver Cell Carcinoma found on the Rainbow Trout, *Salmo irideus* GIBBONS.

(*With 1 Plate and 1 Table*)

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In spite of its frequent occurrence, our knowledge on the carcinoma of the fish liver is rather superficial. So far as I am aware, the disease have been found on three species of fishes. Two cases by TAKAHASHI (1929), and one by HADDOW & BLAKE (1933) recorded it developed in cod fish or rainbow trout (Table). But their studies were somewhat incomplate in histological details.

In September 1953 and January 1954, I was able to examine two cases of liver cell carcinoma on the rainbow trout, *Salmo irideus* GIBBONS, taken by myself from the culture ponds at the Trout Culture Experimental Station at Samegai, Shiga Prefecture, Japan, and to make histo- and cyto-pathological studies of these.

My thanks are due to Professor Y. OKADA, Dean of our Faculty, for his guidance, to Dr. H. HAYASHI of the Faculty of Medicine, for his advices, and to Mr. I. ITO and Mr. T. KAMATA of the Experimental Station at Samegai, who helped me in collecting the materials.

Material and Method

The two specimens examined were both female. One (Case I) was 23 cm. in length and 246 gr. in weight, and retained many dead or collapsed eggs in the body cavity. The other (Case II), the examination of which was made some days after death, was 44 cm. long.

After fixation by 10 per cent aqueous solution of neutral formalin, Bouin's fluid, absolute alcohol, or ca. 5 per cent aqueous solution of gracial acetic acid, tumour pieces together with surrounding tissues were embedded in paraffin, or celloidin, or gelatin (for freezing section method). Sections were cut 5 to 15 μ thick, and stained as control by Ehrlich's hematoxylin and eosin Y, Van Gieson's picro-fuchsin, Weigert's resorcin-fuchsin, Azan's trichrome, or Bielschowsky's silver impregnation method. In histochemical studies, the section were stained Best's carmin for the detection of glycogen, Sudan black B or Sudan IV for fatty substances, 0.1 per cent toluidin blue at pH 7.0, 2.5, and 1.5 for metachromasy of acid polysaccharides, hemosiderin reaction for blood pigments, and Stein's method for the test of bile pigments.

Observation

Case I: Liver tumour, the presence of which was detectable by touch even from the outside of body, was of a growth type composed of two large ovoid swellings occupying posterior two thirds of the liver. It was 5.5 cm. long (3.0 cm. and 2.5 cm), 4.1 cm. wide (4.1 cm. and 3.4 cm.), and 2.8 cm. high. Pressed by residual dead eggs, it showed many hollows and indistinct speckles on the surface. Its ground colour was grayish brown, but was scattered with numerous black of reddish black spots which were visible though the outer covering. The spots were 1.5 mm. or less in diameter (Figs. 1 and 2).

Before going into the desciption of the tumour it may be well to give a short account as regards the structure of the uncancerous portion of the liver. The latter had the external covering tawny in colour and composed of nodules of 2 to 4 mm. in diameter. It was relatively firm and showed a certain resistance in making a cut. The cut surface was also nodular, the nodules being bordered from adjoining ones by yellowish gray septums.

Cut surface made about the middle of the tumour was rather brittle and pale brownish white in colour, including at the same time numerous black or reddish black spots and thick fibre bundles of connective tissue. Periferal parts of the cut surface, had altered into brown or dull yellowish brown colour containing rather scanty spots. Outer covering was rather than in uncancerous portion (Fig. 2).

Gall bladder was very large in size, and dark green in colour owing to oxidation of gall pigments. Both the kidney and the spleen were swollen to some extent due to an edema accompanying deposition of protein granules in the cytoplasm of composing cells.

Metastasis was undiscovered.

The lobules composing the parenchyma of the tumour were divisible into two types according to their histological arrangement. In one type they were greatly enlarged owing to an extraordinary multiplication of constituent cells, while in the other they were collapsed and remained to contain scattering hypertrophized cells. These two types of lobules were intermingled with each other throughout entire parenchyma and intermingling of these appears to suggest the rapidness of the growth of the tumour.

The interstitial tissues showed degeneration to some extent. The hyalinized parts were stained red by Van Gieson's picro-fuchsin, and showed negative metachromatic colour reaction by toluidin blue stain. The greater part of blood vessels except central vein, and of fibrous stroma proliferated by cirrhosis had altered into hyalin by the fusion of connective fibres as the characteristic of carcinogenesis complicated with cirrhosis. Hyalin masses which had appeared black or reddish black spots to the naked eye, contained numerous fine granules

of melanin, larger granules of blood pigments which were positive to hemosiderin reaction, and broken red corpuscles in the deeper interstices. Indistinct speckles seen through external covering may have originated in the hemorrhage within this tissue. Lattice fibre, bile duct and bile capillaries were absent.

The tumour cells were polyhedral and provided with prickle processes characteristic to cancerous cells which were variable in configulation. Generally speaking, these signs of progressive cell anaplasia can be observed more abundantly in the collapsed lobules than in the one where proliferation of cells took place. Moreover, cells in the regions which are rather feeble in circulatory, respiratory, nutritional functions owing to poor blood supply contained bile pigments. Those in the region of hemochromatosis had been blood pigments together with bile pigments. But cytoplasm of all the cells contained neither glycogen nor other sorts of polysaccharides. The protein granules found in the cytoplasm of collapsing lobules had been dissolved by diluted acetic acid.

The nuclei of those cells were at times abnormally enormous in size, or showed another time a distinct hyperchromatosis. They contained again another time a large-sized or destorted nuclei, or other sorts of abnormalities. Abnormalities of cytoplasm and karyoplasm such as those enumerated here, appear to present the earliest indication of the death of the cells. Mitotic figure was rather rarely found and even when discovered, it was abnormal.

Growth of the tumour appears to have been originated in the penetration of cells into the interstices of the surrounding tissues, and of their rapid enlargement within the tissue spaces, or along bile ducts and blood vessels. It may be called therefore infiltration type. The uncancerous portion was attacked with cirrhosis. This portion has entirely altered in its histological structures, beeing largely occupied by broad fibrous bounds. Its connective tissue contained numerous bile ductules, lymphocytes, and was scattered with small nests of hepatic cells. Normality in arrangement of veins was entirely indisorder. Only a few cases, pycnosis and cytoplasmic vacuolization were found, but hyalinization was absent.

Such abnormalities as morphological and pathological natures of the cytoplasm of tumour cells appear to suggest the origin of the disease. The present case, is complately identical with that of malignant hepatoma reported by HADDOW & BLAKE (1933) in the following points: presence of prickle processes characteristic to carcinoma cells, cytoplasmic degeneration in the cells of collapsing lobules, rapidness and infiltration type of growth, or resemblances of tumour structure. From heretofore stated, the present case may be named liver cell carcinoma (malignant hepatoma).

Case II: Examination of the material was made a few days after death. Tumour discovered in the central part of the liver was 6.4 cm. long, 4.9 cm.

wide, and 3.0 cm. high. Post mortal changes due to autolytic digestion of tissues was distinct, and the outer capsule and parenchymatous tissue were damaged. Body cavity was occupied by a brei substance containing of blood, tumour pieces, pus-like substances, and other exudates from the broken part of tumour. In order points, the tumour was very similar to that of the Case I.

Although post mortal histo-dialysis made the microscopical examination unsatisfactory, vigorous prolification of parenchymatous cells, hyalin formation, and the absence of lattice fibre were observed. In the uncancerous portion of the liver, the possibility of cirrhotic abnormalities was indicated by fibrosis, nodular formation, and atrophy of lobules. It appears that in this case the liver has suffered with the same disease as the previous one (Case I).

Table: Known cases of tumours developed in the liver.*)

Species	Tumour name	Identification	Metastasis	Author
Gadus brandti	liver cell carcinoma	multiple growth	absent	TAKAHASHI (1929)
Theragra calcogramma	〃	〃	〃	〃 (〃)
Salmo irideus	〃	single growth	〃	HADDOW & BLAKE (〃)
〃	〃	double growth, complicated with hyalin degeneration, hemochromatosis, and cirrhosis	〃	KUBOTA
〃	〃	same with single growth, and post mortal changes	〃	〃

*) The above table indicates the examples of tumour taken from the records together with two cases in cultured rainbow trout examined by myself. All of the five cases shown in the table belong to the liver cell carcinoma and the cholangioma has never been found on the fish liver.

Summary

The present study dealt with liver cell carcinoma found on two females of the rainbow trout, *Salmo irideus* GIBBONS from the culture ponds at the Trout Culture Experimental Station at Samegai.

In Case I, the tumour tissue consist of a large amount of proliferated parenchymatous cells, rather a few stroma including with blood vessels and others. Hepatic lobules are intermingled with two types as stages of the proliferating, or as it of the collapsing. In the former, the tumour cells are polyhedral in sharp with prickle processes. In the latter they can be observed more abundantly the signs of anaplastic degeneration in this stage than in former.

Tumour is infiltrative growth, and seems to have rapidly grown and may be malignancy. The causal genesis of this tumour is unknown.

In Case II, post mortal changes due to autolytic digestion of tissues have been occurred, and the outer capsule and parenchymatous tissue were damaged. Body cavity was occupied by a brei containing of pus-like and other substances. Post mortal histo-dialysis made the microscopical observation unsatisfactory. But the pathological characteristics of the tumour itself was very similar to those in the Case I.

Of two cases of this disease observed by myself, they have a thing in common which characterized by complication of cirrhosis, absence of lattice fibre, hyalinfomation of connective tissues, large and rapid growth, or absence of metastasis. It may be concluded from the above statement that the original seat of the primary carcinoma in these materials is the parenchyma of liver.

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Explanation of the Plate

Fig. 1. Liver cell carcinoma developed in rainbow trout, *Salmo irideus* GIBBONS. Showing ovoidal like growth together with cirrhotic portion. A: outer view, B: cut surface. Slightly less than natural size.

Fig. 2. Photomicrographs of tumour tissues. Sections showing the multiplication of tumour cells (A) and the parts of hyalin degeneration (A and B). Ehrlich's hematoxylin and eosin Y stain, ca. ×80.

Fig. 3. Tumour cells, showing prickle processes and abnormalities of degenerated cytoplasm. Drawn by Abbe's apparatus.

f—h, n, q, r: including melanin, bile pigments, or blood pigments.

i—n: including oil drops.

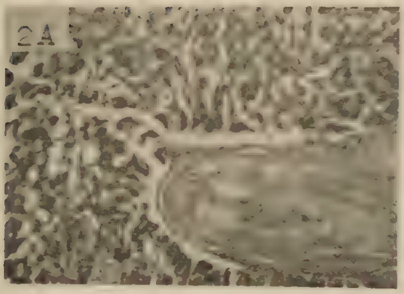
l—s: cloudy swelling.

l—p: edematic cytolysis.

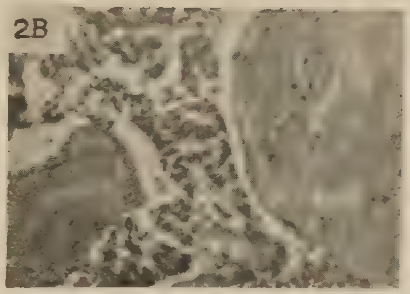
1A

1B

2A



2B



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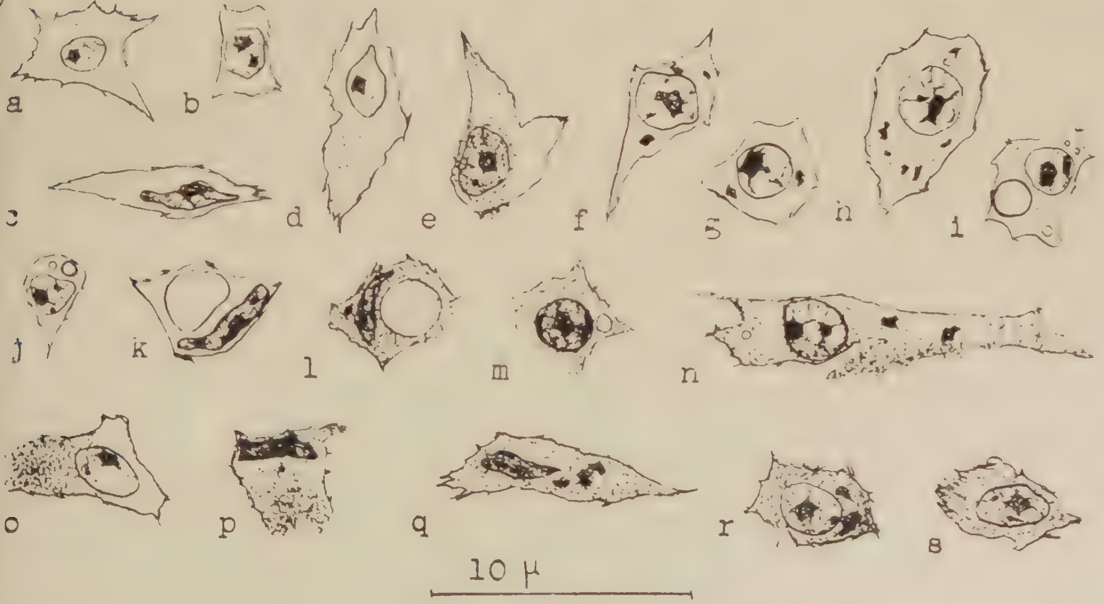

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Studies on Vitamin B_6 in Marine Products II. The Distribution of Vitamin B_6 Group in Aquatic Animals

(*With 2 Figures and 4 Tables*)

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In the previous publication of this series[1], the authors reported the amount of total vitamin B_6 in marine fish, using the microbiological method of Atkin et al.[2]. The present paper deals with the amounts of the three components of vitamin B_6 group in aquatic animals.

To determine each of the three forms of the group—pyridoxamine, pyridoxal, and pyridoxine—either individually or together, the microbiological method of Rabinowitz and Snell[3] has been considered the most suitable. Recently, Fukui[4] improved this method by combining the fractionation by an ion exchange resin with the microbiological means.

Since the authors investigated these original and modified methods comparatively and found that the latter was preferable in feasibility, they carried out present experiments using the latter method which had been modified by the authors and obtained the following results on the distribution of vitamin B_6 group in marine fish, molluscs, and crustaceans.

Experiments

Samples used for assay were caught in the Ise Bay and obtained in fresh conditions through the Tsu Fish Market during the period from November, 1954 to May, 1955. The estimations were carried out in a dark room as possible, since the B_6 group is easy to be broken[5].

1). Extraction of vitamin B_6:

To 5g meat of fish and to each 6g meat of molluscs and crustaceans the distilled water was added up to 500 cc and the whole was homogenized in a Waring Blendor, and after adding 100 cc of 0.11 n H_2SO_4 to 100 cc of above homogenized liquid, it was hydrolyzed for two to three hours at 20 pounds.

It is known that vitamin B_6 neither changes its form nor be destroyed under these conditions[6][7]. After cooling, the filtrate was neutralyzed to about pH 6.5 and made up to a definite volume. This solution was denoted as Fraction A.

2. Separation of pyridoxamine:

Since above extractive contains pyridoxamine, pyridoxal, and pyridoxine, the Na-form of Diaion K (or Duolite C_{60}) 40–60 mesh in size was used for the adsorption of pyridoxamine. After 20 cc of the Fraction A was driven with a speed of 1–2 cc/min through the adsorption tower in which previously about 1.5g of Diaion K was filled up and thoroughly activated by passing on it the solutions of KCl–NaCl, HCl, and NaOH, and the distilled water in order, the content of the tower was washed with 30 cc of the distilled water.

The elucidated and washed extracts were combined together to a definite volume, and the total was denoted as Fraction B.

3). Inactivation of pyridoxal:

The Fraction B contains pyridoxal and pyridoxine. In order to inactivate pyridoxal, 5.0 cc of 5 n NaOH and 5 cc of acetone were added to 20 cc of the Fraction B and the solution left for four hours at room temperature in a dark place. After acetone had been removed by vacuum evaporation, the extract was neutralized with sulfuric acid and made up to a definite volume.

This extract is the Fraction C, in which pyridoxine alone exists.

4). Microbiological assay of vitamin B_6:

The B_6 content in each Fraction was estimated by the microbiological method using *Sacch. carlsbergensis* as mentioned in the previous paper[1], employing a 50cc erlenmeyer flask as culture flask and keeping the culture calm in an incubator at 30°C without vibration. Each Fraction was employed for assay after diluted as adequately as about 5–30 mγ/cc of B_6 to be found.

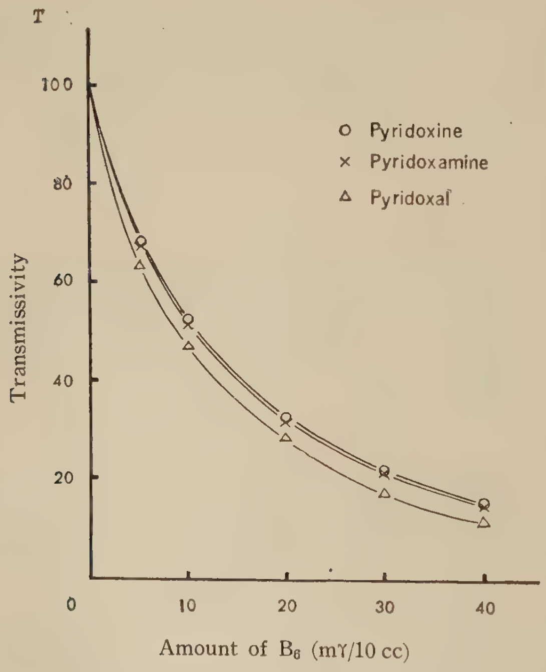

Fig. 1. Standard curves of the three forms of B_6 for *Sacch. carlsbergensis*.

5). Calculation of B_6 group:

Since the response curves of *Sacch. carlsbergensis* for pyridoxamine, pyridoxal, and pyridoxine are different respectively (Fig. 1), each relative curve between the concentration and turbidity of organisms was previously measured, and then each value of three forms of B_6 was obtained calculating as shown in Table 1.

Table 1. Calculation of the three forms of vitamin B_6.

Fraction	Total (Fraction A)	Effluent + Washing (Fraction B)		Aceton – Alkali treated soln. (Fraction C)	
Standard used	Pyridoxamine 2HCl	Pyridoxamine 2HCl	Pyridoxal HCl	Pyridoxal HCl	Pyridoxine HCl
Value measured	a	b	c	d	e

Pyridoxamime 2HCl = a–b
Pyridoxal HCl = c–d
Pyridoxine HCl = e

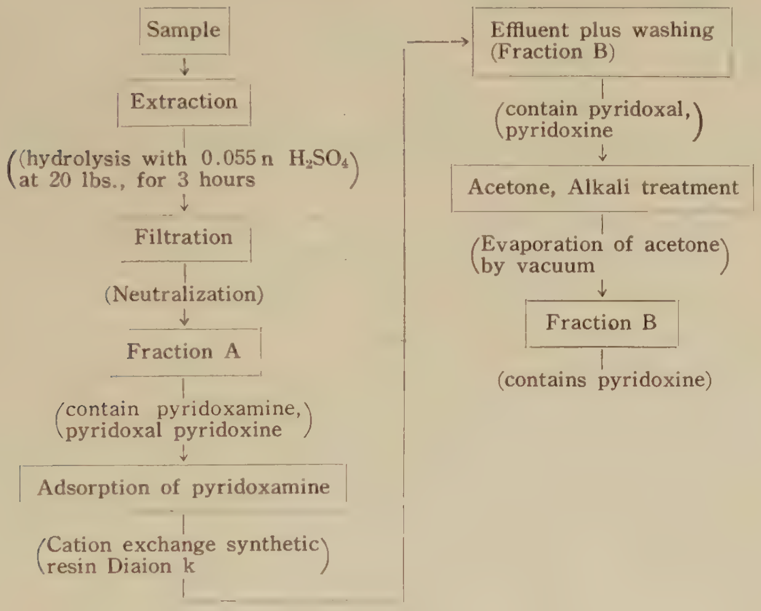

Fig. 2. Procedure of the determination of B_6 group.

Results and Discussion

In the Fukui method, pyridoxamine was adsorbed with a cation exchange resin like Duolite (or KH4B) and eluted into 0.2 n NaOH–nKCl solution at 80°C, then directly estimated by bioassay. However, it was shown that the commercial Duolite C_{60} had a poor elutive power for pyridoxamine, though the adsorption for pyridoxamine was good, in the case when 20 cc sample containing 1.5 mγ pyridoxamine was effluented through 1.5 g Duolite and, after washing with 30 cc of distilled water it was eluted with 0.2 n NaOH–1 n KCl solution at 80°C. None of pyridoxal and pyridoxine were adsorbed with Duolite. Diaion K acted in quite same manner.

The results of the experiment are shown in Table 2.

Table 2. Adsorption of B_6 group with synthetic resin.

Solution tested	Duolite C_{60}–Na form			Diaion K–Na form		
	mγ found in		Adsorption %	mγ found in		Adsorption %
	Effluent	Elute		Effluent	Elute	
Pyridoxamine 2HCl 15 mγ	0		100	0		100
Pyridoxamine 2HCl In NaOH–KCl eluted solution		9.0	40		10.5	30
Pyridoxal 15 mγ	15.0		0	15.0		0
Pyridoxine 15 mγ	15.0		0	19.0		0

From these results, the estimation was carried out in such a way as pyridoxamine in a solution be adsorbed with a ion exchange resin and measured by deducing from initial total B_6 the in effluent residued B_6, expressing B_6 as pyridoxamine in both cases.

This method is at present reasonably satisfactory for determining all three forms, provided adsorption and effluent be adequate.

The distributions of B_6 group in fish, molluscs, and crustaceans indicated by this method are shown in Table 3 and 4.

It was shown that the red meat fish contained more total B_6 than the whites in the same manner as reported in the previous paper.

Hoshizame and Wakasakarei, however, approximately contained equal amounts to red meat fish. Comparing with fish, there were less amounts of total B_6 in molluscs and crustaceans, except in the viscera of clam containing a considerable amount. It has been shown that of the three forms of the vitamin, pyridoxamine was found to be a predominant component in both animal tissues and yeast, while in plant tissues, pyridoxine had a leading position. Similarly in our experiments, pyridoxamine be ranked first among the three, pyridoxal next, and pyridoxine last, and no relation was found on the ratio of the three forms of vitamin B_6 in aquatic animals.

Though in the previous report the amount of total B_6 had been indicated as pyridoxamine, it may be regarded approximate from these experiments to express the total B_6 as pyridoxamine, provided each form of B_6 can't be measured.

It should, however, be attended that since the responses of yeast to pyridoxamine and pyridoxal differ a little as mentioned before, the more the amount of pyridoxal in a sample, the more is the amount of total B_6, which is indicated as pyridoxamine, comparing with the sum of the three forms.

In general, pyridoxamine was the most abundant form in molluscs and crustaceans as in fish, though pyridoxamine and pyridoxal in them were found in the order of their amounts reversely as in fish, and it was found that the distribution of the three forms varied with species.

Table 3. Contents of B_6 group in fish.

Sample	Scientific name	Moisture %	In fresh matter γ/g			In dry matter γ/g			Total B_6 γ/g	
			Pyridoxamine 2HCl	Pyridoxal HCl	Pyridoxine HCl	Pyridoxamine 2HCl	Pyridoxal HCl	Pyridoxine HCl	Fresh matter	Dry matter
Hoshisame	*Cyrrias manazo*	75.66	11.20	1.50	0.15	46.01	6.16	0.61	12.85	52.78
Wakasakarei	*Verasper variegatus*	79.58	5.30	1.21	0.48	25.95	5.93	2.35	6.99	34.23
Honsaba	*Scomber japonicus*	69.33	9.84	0.53	0.48	32.18	1.72	1.56	10.85	35.46
Maaji	*Trachurus japonicus*	75.69	6.0	1.86	0.12	24.68	7.65	4.93	7.98	37.26
Tobiuo	*Cypselurus agoo*	71.18	9.76	1.20	0.78	33.87	4.16	2.70	11.74	40.73
Hoshisame (liver)	*Cyrrias manazo*	39.57	15.72	2.13	0.57	26.01	3.31	0.88	18.42	30.10
Honsaba (liver)	*Scomber japonicus*	69.21	16.84	2.02	0.12	54.68	6.56	0.31	18.98	61.55
Honsaba (testis)	*Scomber jvponicus*	75.65	2.32	0.31	0.06	9.52	1.27	0.24	2.69	11.03
Kurodai	*Sparus macrocephalus*	78.43	3.76	0.42	0.40	17.43	1.94	1.85	4.58	21.22
Hime	*Hime japonica*	97.63	2.56	0.46	0.11	11.86	2.13	0.50	3.13	14.49
Oniokoze	*Inimicus japonicus*	85.19	3.38	0.11	0.06	22.80	0.74	0.40	3.55	23.94
Matodai	*Zeus japonicus*	78.30	4.64	0.25	0.17	21.37	1.15	0.78	5.06	23.30
Bora	*Mugil cephalus*	78.53	6.08	0.72	0.00	28.31	3.35	0.00	6.80	31.66
Akaamadai	*Branchiostegus japonicus*	78.47	1.96	0.36	0.00	9.10	1.67	0.00	2.32	10.77
Yumekasago	*Helicolenus hilgendorfi*	79.44	5.40	0.71	0.27	25.08	3.45	1.31	6.38	28.53
Mejina	*Girella punctata*	76.17	1.12	0.27	0.10	4.69	3.13	0.41	1.49	5.82
Funa	*Cyprinus auratus*	78.81	2.70	0.56	0.28	12.74	2.66	1.32	3.54	16.72
Gotenanago	*Anago anago*	86.10	1.91	0.93	0.30	13.73	6.69	2.15	3.11	22.57
Kijihata	*Epinephelus akaara*	78.22	1.04	0.93	0.38	4.77	4.26	1.74	2.35	10.77

Table 4. Contents of B_6 group in molluscs and crustaceans.

Sample	Scientific name	Moisture %	In fresh matter γ/g			In dry matter γ/g			Total B_6 γ/g	
			Pyridoxamine 2HCl	Pyridoxal HCl	Pyridoxine HCl	Pyridoxamine 2HCl	Pyridoxal HCl	Pyridoxine HCl	Fresh matter	Dry matter
Magaki (viscera)	*Ostrea talienwahnensis*	82.20	0.09	0.25	0.17	0.54	1.44	0.97	0.51	2.95
Hamaguri (viscera)	*Cytherea meretrix*	78.65	9.09	0.12	0.03	42.48	0.59	0.16	9.24	43.23
Bakagai (common meat)	*Mactra sulcatoria*	79.13	0.29	0.07	0.38	1.40	0.35	1.85	0.74	3.60
Sazae (common meat)	*Turbo cornutus*	74.74	0.52	0.12	0.08	2.08	0.50	0.33	0.72	2.91
Yariika (common meat)	*Loligo bleekeri*	74.40	0.34	0.17	0.43	1.34	0.69	1.70	0.94	3.73
Afuriika (common meat)	*Sepiotenthis iessonana*	74.45	0.22	0.00	0.11	0.87	0.00	0.43	0.33	1.30
Ashiaka (viscera)	*Penaeus ashiaka*	78.40	0.55	0.01	0.50	2.57	0.06	2.32	1.06	4.95
Gazami (common meat)	*Neptunus pelagicus*	78.78	1.99	0.07	0.32	9.38	0.34	1.53	2.38	11.25
Namako (connecting tissue)	*Stichopus japonicus*	92.83	1.76	0.00	0.00	10.29	0.00	0.01	1.76	10.30
Namako (viscera)	*Stichopus japonicus*	88.62	3.08	0.00	0.64	14.42	0.00	3.01	3.72	17.43

It may be that these facts are due to the differences in food life and environment of animals.

Since the amounts of the three forms of vitamin B_6 in aquatic animals may be affected by due to age, sex, fishing method, season, freshness, and other environmental factors, the experiments should be carried out with considerations of these points.

Summary

1). First, to determine the three forms of vitamin B_6, we examined the FUKUI method in which after fractionating pyridoxamine with a cation exchange resin each form of the B_6 group is estimated, and then investigated the distribution of B_6 in aquatic animals, using the FUKUI method modifying a little.

2). Pyridoxamine was found to be the most aboundant form in equally fish, molluscs, and crustaceans.

3). Pyridoxal and pyridoxine were found in the order of their amounts in fish, though a reverse relation was shown in molluscs and crustaceans.

The ratio of the three forms of vitamin B_6 containing in molluscs and crustaceans varied with species considerably.

4). The amounts of total B_6 in molluscs and crustaceans were considerably less than those in fish.

We wish to express our thanks to Mr. SUZUKI for his assistance regarding the fish name, and Mr. SAKAMORI (Tsu Fish Market) for kindly supplying the samples.

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Studies on Vitamin B_6 in Marine Products III.
On the Relation between the Production of Vitamin B6 and the Spoilage of Culture Medium by Putrefactive Bacteria in Marine Fish

(*With 5 Tables*)

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Gradually it has been established that the vitamin B_6 group plays a important role on the nitrogen metabolism of almost living matters and especially the enzymatic function of pyridoxal phosphate is connected with many amino acid metabolisms in living matters. The vitamin B_6 is considered to be responsible for the following enzymes—transaminase, racemase of alanine and glutamic acid, amino acid α-decarboxylase, aspartic acid β-decarboxylase, tryptophanase, tryptophane desmolase, kynureninase, desulfhydrase of cystein and homocystein, and histaminase[1][2][3].

Though the chemical changes catalized by these enzymes may appear to have little in common, they provide important and interesting problems on biological chemistry.

Since, from these points the roles of the B_6 group in such reactions as nitrogen metabolism, many phenomena after death, and the biochemical reactions by putrefactive bacteria in aquatic animals, are particularly important, it is a very interesting matter to investigate the various chemical changes in aquatic animals from the stand point in regard to B_6. Especially, it is a noticeable fact from sanitary point of view that though amino acid decarboxylation is not only an important means by which bacteria can metabolize some amino acid but also provides these organisms a means of producing alkaline substances with which they can alter an undesirable acidic environment, there produce several amines toxic for living animals as a result of decarboxylation.

According to many researches[4][5][6] which have been reported on the putrefactive bacteria and their functions, in the earlier course of spoilage of aquatic animals there are considerable number of bacteria which are rather indifferent to spoilage, but there increase a number of non-sporing and gram negative bacteria, which possess a strong putrefactive power, along with the progressive spoilage. Thus, there are various putrefactive bacteria such as active and inactive bacteria in the bacterial group of aquatic animals. This difference in putrefactive powers of bacteria is supposed to be responsible for the B_6-producing

ability, or strictly speaking, for the pyridoxal-phosphate producing ability. Meanwhile, there are such various bacteria as those which need the vitamin B_6 as a growth factor or need no B_6 owing their synthesising abilities, and on these problems there are many reports (THOMPSON[7], HOLDEN and SNEEL[8], HAMADA, SASAKI, and TANAKA[9], BEADLE and TATUM[10], TAKATA et al.[11], LEPKOVSKY[12], GYÖRGY[13], and KUHN[14]). However, there are no reports on the relation of B_6-producing ability of marine putrefactive bacteria with their putrefactive functions, except on the B_6-forming ability of *Pseudomonas fluorescens*.

The authors intended to clarify certain relations between the B_6-producing ability of marine putrefactive bacteria and their putrefactive functions, and found that the bacteria of active B_6-producing ability indicated an active putrefactive function.

Experiments

1) Separation and identification of putrefactive bacteria:

In order to separate the bacteria which are considered to participate in the spoilage of fish, Saba, Guchi, and Samma were left at a room temperature, and putrefied spontaneously, and then the bacteria in various stages of spoilage were isolated by plate culture, using a B_6 free medium (its composition is shown in Table 1) and a ordinary agar in such a way that, after the contaminated bacteria

Table 1. Composition of B_6 free medium

dextrose	5 g	$MnSO_4 \cdot 7H_2O$	5 mg
$CaCl_2 \cdot 2H_2O$	250 mg	Distilled water	1000 cc
$MgSO_4 \cdot 7H_2O$	250 mg	pH	6.5
$FeCl_3$	5 mg		
10 % vitamin free casein hydrolysate			80 cc

were cultured several times on B_6 free medium, a bacterium was purely separated by plate culture of ordinary agar. Separated bacterium was determined by "Bergey's Manual of Determinative Bacteriology (sixth ed.)".

2) Measurement of B_6-producing ability of the separated bacterium:

i) In the case of B_6 free medium:

10 cc of B_6 free medium was taken into a 50 cc erlenmeyer flask, and after the sterilization, from a slant of the culture medium in which the bacteria were previously cultured for 48 hours at 25°C, the bacteria were transfered into the flask with a straight platinum wire and cultured for 48 hours, and then after equal volume of 0.11 n H_2SO_4 was added to the flask, these contents were hydrolysed at 20 pounds for 3 hours, and after neutralization the amounts of total B_6 in a culture medium were estimated by microbiological assay using *Sacch. carlsbergensis*[15].

ii) In the case of Bouillon:

Ordinary Bouillon (4 g of Bonito extract, 6 g of pepton, 1 g of NaCl, and 1000 cc of tap water, pH 6.5) was employed for the cultivation of a separated bacterium, and the determination of total B_6 was done under the same process as in B_6 free medium.

3) Measurement of ability of amino acid decarboxylation:

Separated bacterium, which was cultured on a slant of ordinary agar, was inoculated on a Bouillon (pH 6.5) containing 2 per cent glucose and precultured at 25°C for 24 hours, then after washing three times with a steriled water in a centrifuge, the activity of amino acid decarboxylase existing in it was measured by the method of LAGERBORG et al.[16], using Warburg's apparatus. In short, to 0.5 cc of M/30 amino acid solution added 1.0 cc of phosphate buffer (pH 5.5) and 1.0 cc of washed suspension of separated bacterium, and the amount of CO_2 evolved for 1 hour at 37.5°C was measured.

4) Measurement of deamination:

Separated bacterium which was precultured on a slant of ordinary agar, was inoculated with a straight Pt wire on 20 cc ordinary Bouillon, and after culturing for 48 hours at 25°C, the volatile basic nitrogen was measured by the aeration method (modified A.O.A.C. method).

Results and Discussion

1) Bacteria which participate in the putrefaction of marine products:

The behaviours of bacteria separated from the various stages of spoilage of Saba, Guchi, and Samma, are shown in Table 2. Symbol A denotes a series of bacteria which were separated from the B_6 free medium, while symbol B denotes those from ordinary agar. Of the 40 strains which were separated, 24 strains of *Pseudomonas*, 5 of *Flavobacterium*, 5 of *Micrococcus*, 5 of *Achomobacter*, and 1 of *Alcaligenes* were found. Of these organisms, those which were separated using B_6 free medium belonged to *Pseudomonas*. These organisms except *Alcaligenes* are familiar in ordinary spoilage of fish.

2) Synthesis of vitamin B_6 by separated bacteria:

The amounts of B_6 synthesized by organisms which were cultured on B_6 free medium and Bouillon for 48 hours at 25°C are shown in Table 3 (the amount of total B_6 was indicated as pyridoxamine). Though synthesized B_6 was considered to be secreted into inner parts of body and the culture medium (the mutual relation of the above two amounts will be dealt with next report), the B_6-producing ability of a separated bacterium was indicated by the amounts of total B_6 which are expressed as pyridoxamine.

Organisms separated from B_6 free medium possessed the B_6-forming ability and produced 4~100 mγ/cc B_6. Though all *Pseudomonas* grew fully and indicated active production of B_6, the organisms belonging to the other genus did not

Table 2. Genus name and

Symbol of strain	Form	Spore	Gram stain	Starch	Gelatin	NO_2	H_2S
A 1		–	–	+	+	+	–
A 2		–	–	+	+	–	–
A 3		–	–	–	–	–	–
A 4		–	–	+	+	+	+
A 5		–	–	+	+	–	–
A 6		–	–	+	+	–	–
A 7		–	–	+	+	–	–
A 8		–	–	–	–	–	–
B 1		–	–	–	–	+	–
B 2		–	–	–	–	+	–
B 3		–	–	–	+	–	–
B 4		–	+	+	–	–	–
B 5		–	+	+	–	+	–
B 6		–	–	+	+	–	–
B 7		–	+	–	–	+	–
B 8		–	–	–	–	+	–
B 9		–	+	+	–	–	–
B 10		–	–	–	–	+	–
B 11		–	–	+	+	–	–
B 12		–	+	–	–	+	–
B 13		–	–	+	+	–	–
B 14		–	–	+	+	+	+
B 15		–	–	+	–	+	–
B 16		–	–	–	+	–	+
B 17		–	+	+	–	+	–
B 18		–	–	–	–	+	–
B 19		–	–	+	+	–	–
B 20		–	–	–	–	–	–
B 21		–	–	–	+	+	–
B 22		–	–	+	–	–	–
B 23		–	–	+	+	–	–
B 24		–	–	+	–	+	–
B 25		–	–	+	+	–	–
B 26		–	–	–	–	+	–
B 27		–	–	+	–	+	–
B 28		–	–	+	–	+	+
B 29		–	–	–	–	–	–
B 30		–	–	+	–	–	–
B 31		–	–	+	+	+	+
B 32		–	–	+	+	–	–

A =Series of strains separated with B_6 free medium
B =Series of strains separated with ordinary agar medium
+ =Positive
– =Negative

characters of separated strain

Litmus milk	Indol	Acetoin	Glucose CO_2	Agar slant pigment	Genus name
acid	–	–	–	greenish yellow fluorescent	*Pseudomonas*
reduce	–	–	–	pale yellow	〃
acid	–	–	–	〃	〃
reduce	–	–	–	〃	〃
–	–	–	–	greenish yellow fluorescent	〃
–	–	–	–	〃	〃
reduce	–	–	–	〃	〃
acid peptonized	–	–	–	〃	〃
–	–	–	–	〃	〃
–	–	–	–	white	*Achromobacter*
acid peptonized	–	–	–	greenish yellow fluorescent	*Pseudomonas*
–	–	–	–	yellow	*Micrococcus*
–	–	–	–	whitish yellow	〃
acid reduce	+	–	–	brownish yellow	*Flavobacterium*
–	–	–	–	light pink	〃
–	–	–	–	white	*Achromobacter*
acid	–	–	–	white	*Micrococcus*
–	–	–	–	whitish yellow	*Flavobacterium*
reduce peptonized	–	–	–	greenish yellow fluorescent	*Pseudomonas*
–	–	–	–	brownish yellow	*Micrococcus*
acid	–	–	–	white to buff	*Achromobacter*
reduce	–	–	–	pale yellow	*Pseudomonas*
acid	–	+	+	white	*Achromobacter*
peptonized	–	–	–	pale yellow	*Pseudomonas*
acid	–	–	–	light pink	*Micrococcus*
acid peptonized	–	+	–	white to buff	*Achromobacter*
acid peptonized	–	–	–	greenish yellow	*Pseudomonas*
–	–	–	–	greenish yellow fluorescent	〃
acid peptonized	–	–	–	〃	〃
–	–	–	–	〃	〃
acid	–	–	–	light yellow	*Flavobacterium*
–	–	–	–	greenish yellow fluorescent	*Pseudomonas*
–	–	–	–	〃	〃
–	–	–	–	〃	〃
–	–	–	–	〃	〃
alkali	–	–	–	light white to yellow	*Alcaligenes*
–	–	–	–	greenish yellow fluorescent	*Pseudomonas*
–	–	–	–	〃	〃
acid	–	+	–	yellow	*Flavobacterium*
reduce	–	–	–	greenish yellow fluorescent	*Pseudomonas*

Table 3. Biosynthesis of B_6 by separated strain
(25°C, 48 hr incubation)

Strain	B_6 free medium		Bouillon		
	Growth	B_6 mγ/cc	Growth	B_6 mγ/cc	Synthesized B_6 mγ/cc
A 1 (P)	+++	76.25	+++	99.9	+35.0
A 2 (P)	+++	79.3	+++	94.4	+29.5
A 3 (P)	+++	44.3	+++	104.65	+39.75
A 4 (P)	+++	58.75	+++	99.9	+35.0
A 5 (P)	+++	77.0	+++	111.1	+46.2
A 6 (P)	+++	94.1	+++	96.8	+31.9
A 7 (P)	+++	94.1	+++	103.0	+38.1
A 8 (P)	+++	99.8	+++	103.5	+38.6
B 1 (P)	+++	48.1	+++	77.9	+13.0
B 2 (A)	+	12.5	+++	82.9	+18.0
B 3 (P)	+++	50.0	+++	108.9	+44.0
B 4 (M)	+	12.8	+++	85.9	+21.0
B 5 (M)	+++	21.9	+++	113.9	+49.0
B 6 (F)	+	24.0	+++	88.1	+23.3
B 7 (P)	+	8.4	+	54.4	−10.0
B 8 (A)	+++	34.0	+++	85.9	+21.0
B 9 (M)	+	10.0	+++	74.8	+ 9.9
B 10 (F)	+	18.7	+++	74.7	+ 9.8
B 11 (P)	+++	46.2	+++	136.9	+72.0
B 12 (M)	+	17.1	+++	66.1	+ 1.2
B 13 (A)	+++	58.1	+++	116.4	+51.5
B 14 (P)	+++	41.2	+++	129.8	+64.9
B 15 (A)	+++	11.0	+++	85.8	+20.9
B 16 (P)	+++	52.3	+++	82.5	+17.6
B 17 (M)	+	4.4	++	68.9	+ 4.0
B 18 (A)	+	11.6	++	57.2	− 7.7
B 19 (P)	+++	70.4	+++	96.8	+31.9
B 20 (P)	+++	48.0	+++	101.1	+35.2
B 21 (P)	+++	40.4	+++	97.9	+33.0
B 22 (P)	+++	50.6	+++	111.1	+46.2
B 23 (F)	+++	88.0	+++	101.2	+36.3
B 24 (P)	+++	80.2	+++	107.0	+42.1
B 25 (P)	+++	30.3	+++	69.3	+ 4.4
B 26 (P)	+++	80.5	+++	112.4	+47.5
B 27 (P)	+++	51.48	+++	100.1	+35.2
B 28 (Al)	+++	33.0	+++	118.8	+53.9
B 29 (P)	+++	80.9	+++	104.5	+39.6
B 30 (P)	+++	30.5	+++	118.8	+53.9
B 31 (F)	+	16.7	++	83.6	+18.7
B 32 (P)	+++	82.7	+++	113.6	+48.7
Control		0		64.9	

(P) = *Pseudomonas* (M) = *Micrococcus*
(F) = *Flavobacterium* (Al) = *Alcaligenes*
(A) = *Achromobacter*

grow sufficiently, and as a result, their abilities of producing B_6 were inactive. Organisms separated from Bouillon showed the increase and decrease of amounts of B_6—+72～ −7 mγ/cc—compared with the initial amounts existing in

Bouillon, and in general, those which indicating few productions of B_6 were the undeveloped, with a few exceptions in which good developments were observed.

For the reason that the amounts of B_6 increased, though it was observed that the organisms separated using B_6 free medium possessed more active power for the production of B_6 than those separated using Bouillon, it might be considered that since the B_6 free medium had glucose, the organisms like *Pseudomonas* might indicate a good development, and that since B_6 was originated in culture medium the B_6 production might become weak. In general, however, it might be shown that the strains which were active for B_6 production in the B_6 free medium indicated active B_6 production in Bouillon similarly. Not only the strains separated using the B_6 free media indicated active production but also *Pseudomonas* separated using the ordinary agar indicated active B_6 production equivalent to the above strains. Fukui[11] investigated the B_6-forming abilities of yeast and fungi imperfect in which it was shown that *Monillia candida* produced 809γ/l of B_6, *Torula utilis* 682γ/l, and American yeast 342γ/l. Compared with these results, the organisms separated by the authors indicated very small amounts, and since the difference was considered to depend upon the period of cultivation, culture medium, and etc., these points will be investigated in next report.

On the B_6-forming abilities and genus of bacteria, it was found that all *Pseudomonas* possessed a superior ability for B_6 production, that *Micrococcus* and *Flavobacterium* except one strain were inferior to the former, and that *Achromobacter* was superior to *Flavobacterium*. In short, from the ability of B_6-production it might be said that there were *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Micrococcus* in order. However, it is out of question that the ability differs from the strains even in same genus. *Alcaligenes* might be said to be active, though it was not proper to say thus from only one example. Considering from above B_6-forming abilities of separated strains, it is supposed that the amounts of B_6 in marine products may increase as the freshness of the marine products falls and the bacteria multiply on them.

3) Putrefaction by separated bacteria:

Putrefaction is thought to be the disintegrative process which is carried on successively in a living matter, and therefore includes very complex biochemical changes. Consequently, though on account of its complexity it is a matter of difficulty to discuss the putrefactive abilities of separated strains, the authors intended to study first on the amino acid decarboxylation and deamination, which are considered to be the most important reactions in the putrefaction, and found out their responsibilities to B_6-forming abilities.

i) Amino acid decarboxylase:

It is known that bacteria possess the α-decarboxlase which produces corresponding amines from six amino acids, 1-arginine, 1-tyrosine, 1-lysine, 1-

Table 4. Volume of CO_2 produced from amino acids by separated strain
(CO_2 μ l/dry bacteria mg/hr, at 37.5°C)

Strain	pH, After incubation	Arginine	Tyrosine	Lysine	Histidine	Ornithine	Glvtamic acid
A 1 (P)*	4.4	13.77			5.45	11.22	
A 2 (P)*	4.5	16.53					
A 3 (P)*	5.3	21.96	13.44				22.03
A 4 (P)*	4.6	18.96					23.03
A 5 (P)*	6.0				7.27		7.69
A 6 (P)	4.8	27.54	14.96			9.35	7.34
A 7 (P)*	4.8	11.01	15.45	22.58			11.01
A 8 (P)*	5.2			3.76	20.01		
B 1 (P)	4.2	18.36	13.09	3.76	23.65	5.61	5.60
B 2 (A)′	5.0						
B 3 (P)*	4.8			11.29			7.34
B 4 (M)′	5.0						
B 5 (M)	5.3		22.44		17.46		
B 6 (F)′	5.4						
B 7 (F)	4.7						
B 8 (A)	6.3						
B 9 (M)′	5.2						
B 10 (F)	5.1						
B 11 (P)*	5.0		12.24		21.83		7.01
B 12 (M)′	4.7						
B 13 (A)*	4.0		11.62				11.01
B 14 (P)	6.4						7.65
B 15 (A)	4.5						
B 16 (P)	4.5						
B 17 (M)′	4.5						
B 18 (A)′	4.5						
B 19 (P)	5.6				14.69		3.82
B 20 (P)*	4.9	5.68		7.52			3.82
B 21 (P)	4.6						5.50
B 22 (P)*	5.3				3.17		3.82
B 23 (F)*	5.0	27.51	20.58				
B 24 (P)*	4.3	14.69					
B 25 (P)	4.4						
B 26 (P)*	4.5	20.22					22.02
B 27 (P)*	4.7				22.03		19.12
B 28 (Al)	5.4	20.38					22.02
B 29 (P)*	5.1		11.22		10.91		
B 30 (P)	6.0						
B 31 (F)′	4.7		14.96				
B 32 (P)*	4.6						

*=Biosynthesis of B_6 is active ′=Biosynthesis of B_6 is poor
Other symbols are same as before

histidine, and 1-glutamic acid[17]. The results we studied on decarboxylases in separated strains are shown in Table 4.

According to this, the degree of amount of decarboxylase may be shown in the following order, those for glutamic acid and arginine, those for histidine and tyrosine, and those for ornithine and lysine.

Concerning to the genera and the presence of decarboxylase, it was found that 19 strains in 24 strains of *Pseudomonas*, 1 in 5 *Achromobacter*, 2 in 5 *Flavobacterium*, and 1 in 5 *Micrococcus* possessed the decarboxylase. *Pseudomonas* indicated widest distribution of decarboxylase.

Table 5. Amount of volatile basic nitrogen produced in Bouillon by separated strain (25°C, 48 hr incubation)

Strain	Growth	pH, After incubation	V.B.N. Produced (mg/20 cc)	Strain	Growth	pH, After incubation	V.B.N. Produced (mg/20 cc)
A 1 (P)*	+++	8.3	+1.959	B 14 (P)	+++	8.4	+4.105
A 2 (P)*	+++	8.4	+3.097	B 15 (A)	+++	8.3	+1.623
A 3 (P)*	+++	7.4	+0.895	B 16 (P)	+++	8.3	+1.922
A 4 (P)*	+++	8.4	+3.377	B 17 (M)′	+++	6.5	+0.093
A 5 (P)*	+++	8.4	+4.683	B 18 (A)′	+++	7.0	+0.329
A 6 (P)	+++	8.4	+4.721	B 19 (P)	+++	8.3	+3.153
A 7 (P)*	+++	8.3	+2.780	B 20 (P)	+++	8.4	+2.743
A 8 (P)*	+++	8.4	+4.161	B 21 (P)	+++	8.3	+1.325
B 1 (P)	+++	8.4	+3.340	B 22 (P)*	+++	8.4	+4.430
B 2 (A)′	+++	7.3	+0.485	B 23 (F)*	+++	8.4	+3.657
B 3 (P)*	+++	8.4	+3.377	B 24 (P)*	+++	8.3	+2.052
B 4 (M)′	+++	6.5	+0.205	B 25 (P)	+++	8.3	+2.500
B 5 (M)	+++	7.0	+0.440	B 26 (P)*	+++	8.4	+3.172
B 6 (F)′	+++	8.2	+1.530	B 27 (P)*	+++	8.4	+3.004
B 7 (F)	+	6.5	+0.112	B 28 (Al)	+++	8.3	+2.239
B 8 (A)	+++	7.0	+0.466	B 29 (P)*	+++	8.4	+3.788
B 9 (M)′	+++	6.7	+0.560	B 30 (P)	+++	8.4	+4.105
B 10 (F)	+++	7.0	+0.429	B 31 (F)′	+++	8.3	+3.676
B 11 (P)*	+++	8.4	+5.299	B 32 (P)*	+++	8.3	+2.780
B 12 (M)′	+++	6.7	+0.149	Control	+++	6.5	1.194
B 13 (A)*	+++	8.4	+3.694				

V.B.N.=Volatile basic nitrogen
Other symbols are same as before

Concerning to the B_6-forming abilities of organisms and their decarboxylations, it was found that almost of the organisms indicating active production of B_6 possessed the ability of decarboxylation.

It will be studied whether the amounts of total B_6 produced in a organism are proportional to the amounts of pyridoxal phosphate produced in it, since the

pyridoxal phosphate is a constituent of decarboxylase coenzyme.

ii) Deamination:

The putrefactive power of a organism may be indicated directly by the amount of volatile basic nitrogen evolved, although the volatile basic nitrogen from a Bouillon in which a separated putrefactive organism was cultured is not always considered to be produced by the deamination of amino acid. The volatile basic nitrogen evolved by culturing a separated organism on a Bouillon for 48 hours at 25°C is shown in Table 5.

From these results, it is shown that *Pseudomonas* possessed the activest deamination, and next *Flavobacterium* and third *Achromobacter*; and that all strains which possessed active B_6-forming function indicated a remarkable increase of volatile basic nitrogen.

As a result of experiments (i, ii), it can be considered that there is a close relation to the B_6-forming ability and the putrefactive function of separated bacteria.

Summary

Since the B_6-enzyme has the important roles in many metabolisms, the authors intended to clarify certain relations between the B_6-producing ability and putrefactive function of bacteria developed on marine products, and separated 40 strains of bacteria in the course of spontaneous spoilages of Saba, Guchi, and Samma, accompanied by the following results:

1) Separated organisms were 24 strains of *Pseudomonas*, 5 of *Flavobacterium*, 5 of *Achromobacter*, 5 of *Micrococcus*, and 1 of *Alcaligenes*.

2) Separated organisms, with few exceptions, indicated good development on B_6 free medium and Bouillon, and the strains except the two possessed the B_6-producing ability. The degree of the ability was shown in the following order; *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Micrococcus*.

3) Amino acid decarboxylation was proportioned to the B_6 production of the strain. The amino acid decarboxylation of *Pseudomonas* was the most powerful and its decarboxylases for arginine and glutamic acid were found in comparative richness.

4) When the separated strain was inoculated to Bouillon, in the strains of the powerful production of B_6 the evolved amount of volatile basic nitrogen was large.

5) From the above experiments it was proved that the putrefaction in marine products was proportioned to the B_6 production of strains.

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Copepods Parasitic on Japanese Fishes

8. The Anthosomidae

(*With 7 Text-figures*)

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The present paper deals with four species of parasitic copepods belonging to the family Anthosomidae and comprises *Anthosoma crassum* (ABILDGAARD), *Lernanthropus atrox* HELLER, *L. chrysophrys* SHISHIDO and *L. shishidoi* nom. nov. (=*L. mugilii* SHISHIDO). These have repeatedly been recorded from our coast, but there are some inconsistencies among authors in their descriptions. *L. shishidoi* nom. nov. is a new name to be proposed for SHISHIDO's (1898) species which has been known by the name of *mugilii*. Since this becomes a homonym of BRIAN's (1898) *L. mugilis* published in the same year, it appears to me desirable that a new name is suggested for either one of these in order to avoid a confusion. As regards this point a remark will be made later*.

Genus *Anthosoma* LEACH, 1816

Anthosoma crassum (ABILDGAARD, 1794)

(Figs. 1 & 2)

Caligus crassus, ABILDGAARD 1794 (after WILSON).
Caligus imbricatus, RISSO 1816 (after WILSON).
Anthosoma smithi, LEACH 1816; KRφYER 1838 (after WILSON); MILNE-EDWARDS 1840, p. 483, pl. 39, fig. 5; BAIRD 1850; KIRK 1887, pp. 31–33, pl. 6.
Otrophesia imbricata, RISSO 1826 (after WILSON).
Anthosoma crassum, GOULD 1841 (after WILSON); STEENSTRUP & LÜTKEN 1861, p. 397, pl. 12, fig. 24; OLSSON, 1869, p. 23; RATHBUN 1884, p. 490; THOMSON 1889, pp. 365–366, pl. 27, fig. 3; BASSETT-SMITH 1899, pp. 468-469; BRIAN 1906, pp. 62–63, pl. 3, fig. 1; T. SCOTT 1905, pp. 112–113, pl. 5, figs. 15–16; T. & A. SCOTT 1913, pp. 108–110, pl. 23, figs. 5–6; ISHII 1916, pp. 147–149; WILSON 1922, pp. 23–26, pl. 1, figs. 1–8; 1923, p. 13; 1932, pp. 446–447, fig. 281; YAMAGUTI 1936, pp. 12–13; DEBOUTTEVILLE 1948, pp. 446–447; DEBOUTTEVILLE & NUNES 1954, p. 205.

Occurrence: 3 females and 1 male obtained in buccal cavity of *Prionace glauca* (LINNÉ), off Tyôsi, Tiba Prefecture, June 9, 1943, by myself; 3 females and 2 males in buccal cavity of *Isuropsis glauca* (MÜLLER & HENLE), off Owase, Mie Prefecture, December 8, 1952, by myself; 1 female and 1 male among teeth of *Heptranchias perlo* (BONNATÉRRE), off Kannoura, Kôti Prefecture, 500 m. deep,

* This is a part of the results of the studies enabled by the financial aid from the Scientific Research Fund of the Ministry of Education.

June 27, 1944, by Dr. Y. OKADA; 5 females and 1 male in the buccal cavity of *Lamna nasus* (BONNATÉRRE), off Kesennuma, Miyagi Prefecture, August 27, 1954, by Mr. A. MATII.

Distribution: Cosmopolitan. European Seas (ABILDGAARD, LEACH, KRØYER) (after WILSON), Öresund (STEENSTRUP & LÜTKEN), British Isles (SCOTT), Mediterranean (BRIAN), Port Natal (S. Africa) (WILSON), Massachusetts (RATHBUN, WILSON), Vancouver Is., California coast (WILSON), New Zealand (THOMSON).

Distribution in Japan: Tôkyô Market (ISHII), Toyama Bay (YAMAGUTI), unknown locality (WILSON).

Host: *Lamna cornubica, Isurus punctatus, I. tigris, I. oxyrhynchus, I. glaucus, I. nasus, Oxyrhina spallanzanii, Carcharias littoralis*, and *Squalus acanthias*. Fastening on to throat, gill arches, or inner side of jaws.

The present species is easily recognized by the peculiar, strongly chitinized, conical carapace and by the appendages which are most complicatedly constructed of all the caligoid copepods. As this species has repeatedly been described and figured, I confine myself to insert the illustrations of the both sexes and of their appendages in addition to giving some accounts on the points about which authors are inconsistent with one another.

Female: Body somewhat curved ventrally, length (of female shown in Fig. 1, B) 11.5, carapace 8.3×5.8 (all in mm). Carapace rather variable in depth of color. Female of lighter color yellowish white, with central part only slightly darker. In deeply colored female, carapace greenish brown, darker forwards, becoming paler and yellowish towards edges. Dark central area flowing backwards in several divergent striations, with cluster of brownish black patches, besides bearing brighter patterns amidst dark area. Dorsal elytra and foliaceous legs grayish white and covered with many translucent spots. Free thoracic segments and abdomen dark yellowish brown; genital segment scattered with black-colored patterns on ventral surface, especially on small areas at posterior end where it articulates with abdomen. Egg strings very long, several times as long as body and containing a series of flattened eggs variable in color from brownish to yellowish according to developmental stages.

WILSON[19] says that carapace of older female shows a postero-median notch. Presence or absence of this notch is merely due to individual variation in my opinion. Carapace bears on either side close to frontal end a notch where it has a small, subtriangular flap. WILSON[19] and YAMAGUTI[22] recognize the flap, but SCOTT T. & A.[15] and ISHII[9] do not. The first named author states that "when the antenna is folded back against the carapace this process is nearly concealed, but it stands out prominently if the antenna be turned forward at right angles to the body axis." Such a statement may be ambiguous and in-

Fig. 1. *Anthosoma crassum* (Abildgaard).

A, female, dorsal view; B, another female, ventro-lateral view; C, posterior part of female shown in B, with thoracic legs of one side removed, ventral view; D, male, dorsal view; E, same, lateral view; F, posterior part of body of same, with thoracic legs of one side removed, lateral view. A ×5.7, B, C, E ×7.7, D ×6.3, F ×7.3.

terpreted in two ways: in one that the flaps are fastened to the antennae, and in the other that they are movably articulated with the carapace. But neither is the case. Flaps are firmly and immovably attached to the carapace, being mere processes of it.

Number of joints composing the 1st antennae is different according to authors. Ishii[9], Wilson[19], and Yamaguti[22] speak of it as 7, while Scott, T. & A.[15] and Wilson[20] as 6. According to my observation, basal joint has on the inner border about the middle a small protuberance from which a shallow groove runs outwards and obliquely proximad over and across the surface, so that the joint appears as if subdivided. It may be ascribable to this fact that the antennae have been interpreted erroneously as 7-jointed, but in reality these are 6-jointed.

Another disagreement exists regarding the structure of the 2nd antennae. In the figures given by authors, the appendages are portrayed sometimes long and sometimes short. Ishii[9] says these are 6-jointed. Nevertheless, according to Scott, T. & A.[15], these "are stout, 3-jointed, longer than the antennules, and armed with strong terminal hook-like claws". Similarly, Wilson[19] states that "they are 3-jointed, the basal joint much swollen, the 2nd joint narrowed distally and armed on the ventral surface with a stout peg so placed as to interlock with the tip of terminal claw". Thomson[18] is of the same opinion, but inserts a better figure. All of them, however, do not note the retractile nature of this pair of antennae. According to my observation, these are usually retracted, when alive, into fleshy sacs on the ventral surface of carapace. If one tries to detach the animal from the host, the antennae are forced to emerge from their sacs on account of their firm clasping of host skin. In accordance with the extent of their emergence at the time when the animal was thrown into the preservatives, their length and then the number of visible joints become variable. The antennae are 5-jointed. Basal joints which form together an elongate rod are retracted in the fleshy depression found close to the anterior end of head by action of strong muscles, while the terminal 3 joints remain exposed. The former joints are somewhat less strongly chitinized than the latter joints, and yet provided with strong musculature within. Structure of apical 3 joints is as described by Wilson[19], but it may be well to add that the terminal claw bears a minute accessory spinule at about the middle of its length. Planes of articulation between every 2 of the exposed joints are strongly oblique to the axis of the antenna, so that the relative lengths of consecutive segments become different between on the outside and on the inside. A twisted appearance of the antennae figured by some of the authors appears to be resulted from this fact.

Palp of the 2nd maxillae (1st maxillae of Wilson) is biarticulated and

tipped with 2 spinules, but the articulation has been overlooked by WILSON[19].

As regards the 1st maxillipeds the named author[19] considers them as 4-jointed, believing geniculation to intervene between the 2nd and the 3rd joints. In his figure of the named limb (Pl. 1, fig. 6), he shows an oblique line on the portion proximad to geniculation. Despite my careful examination, I am unable to find any subdivision of this portion into joints. It appears that he has mistaken the strong surface ribs for line of articulation. He regards, further, the distal joint as the 3rd and the apical appendix which is, with all probabilities, corresponding to the flagellum of other caligoid copepods as the 4th. So far as I am aware, ISHII[9] gives the most excellent figure about this appendage. His treatise appears to have escaped the notice of the American author, since it has been published in Japanese language (in Dobutsugaku-Zassi). According to my observation, the appendage in question shows following structure: It is 2-jointed, being composed of broad proximal and narrow distal joints; the latter less than half the width of the former and carrying at apex a movably articulated knob-like appendix. Proximal joint traversed with strong ribs on its surface. Outer margin of distal joint flattened to form a narrow, crescent, longitudinal lamella and produced at end into a short finger-like process; apex truncate, forming a flat circular disc which is circumscribed by short acuminate denticles and bears a bundle of thick setae on the inside. Terminal knob hemispherical, its flat side rather feebly chitinized, but rich in flexibility so as to be folded against truncate end of distal joint, and surrounded by a row of sharp denticles which closely interlock with those on that joint providing a good means to seize host skin or others. Other side of the knob convex, traversed with longitudinal striations on its surface.

Palm of 2nd maxillipeds has the margin upon which claw closes grooved and carries irregularly shaped ridges on both sides of the groove, whose proximal end forms a low truncate process, surmounted with a short spinule.

As regards the constitution of thoracic segments and their legs a certain misleading has been prevailed. First segment is fused with carapace, though boundary is indicated ventrally by a transverse fold of surface. Second to 3rd segments very short. Elytra (dorsal plates) arise from 2nd (1st free) segment and never from 4th as WILSON[19,22] and others believe. Third segment narrower than either of 2nd and genital segments. Fourth and genital segments fused together to form an enormous segment which is swollen dorso-ventrally, barrel-shaped, and with widely rounded outlines. First pair of legs ovoid, with a distinct notch on inner margin. Second nearly quadrangular, with acute external and posterior corners as well as with inner notch. Third resembling 2nd, but without notch. Fourth lacking.

Male: Crooked usually more strongly than in female and smaller in size,

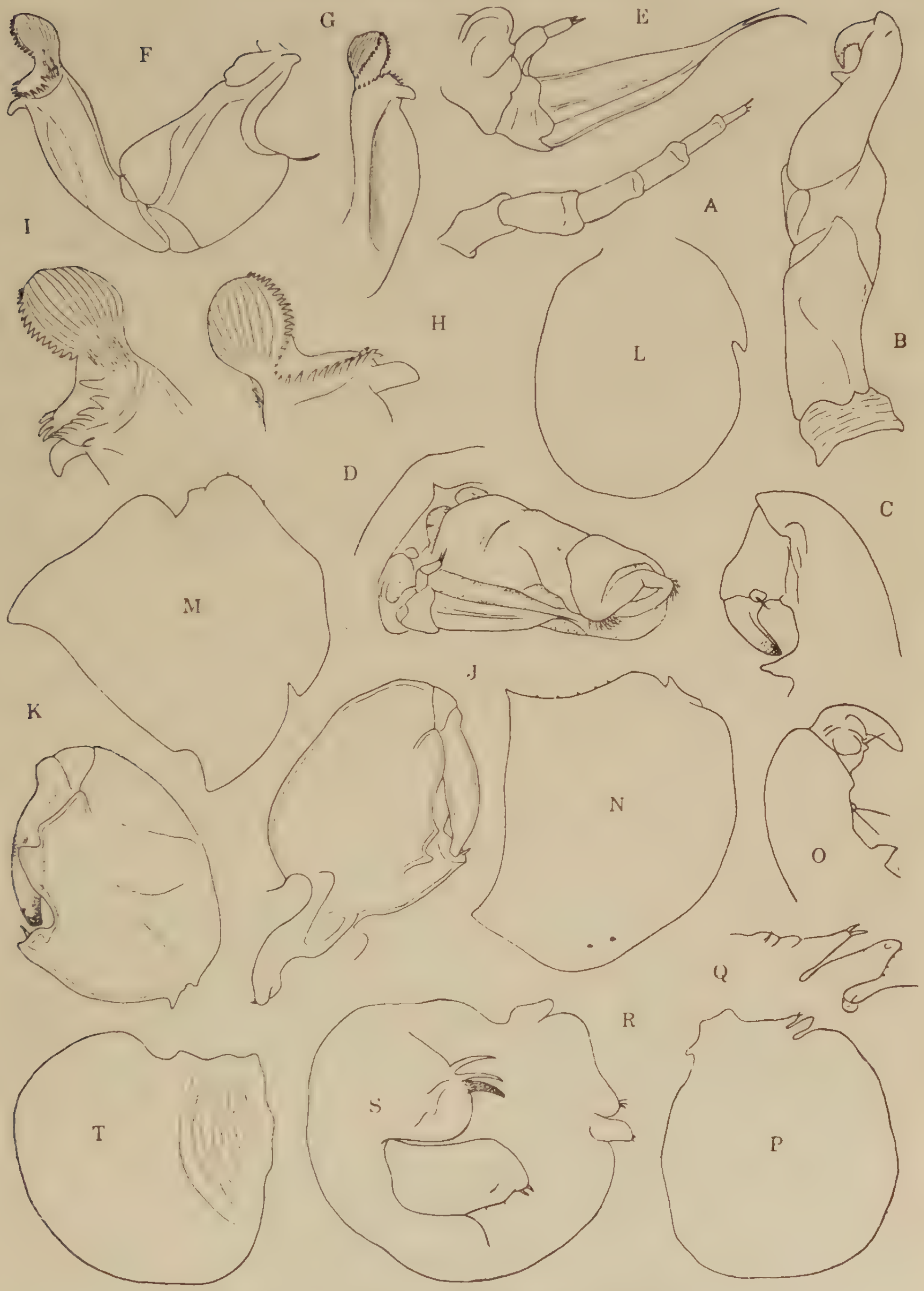

Fig. 2. *Anthosoma crassum* (ABILDGAARD).

A–N, female; A, 1st antenna; B, 2nd antenna; C, tip of same, further enlarged; D, mouth tube and 2nd maxilla; E, 2nd maxilla; F, 1st maxilliped, outer surface, G, same, apical joint, inner view; H, apical knob of same, inner view; I, same, another view; J, 2nd maxilliped, posterior view; K, same, anterior view; L, 1st swimming leg; M, 2nd leg; N, 3rd leg; O–T, male; O, tip of 2nd antenna; P, 1st swimming leg; Q, basal lobes of same, further enlarged; R, 2nd legs; S, ramus of same, further enlarged; T, 3rd leg. A, B, J, K, P, R, T ×12, C, E ×27, D, F, H ×18.5, G, I, Q–S ×37, L–N ×11, O ×34.

but with free segments proportionally longer; length 9.05 as body is curved, carapace 7.1×4.7 (all in mm). Carapace same as that of female in color; free segments colored in brownish black, narrow inter-segmental areas, posterior faces of dorsal carinae, and longitudinal lines along lateral sides of segments much paler in color, being nearly whitish.

Carapace as that of female, with or without postero-median notch. Appendages belonging to cephalothorax same in constitution as those of that sex except 2nd antennae. Terminal claw of 2nd antennae with a short falciform and a rounded protuberances close to the base, besides with a short seta; succeeding joint with 2 similar setae on inner margin in the region between apex and acuminate process against which tip of claw acts.

First thoracic segment entirely fused with carapace not to retain any sign of boundary. Following segments gradually narrower from before backwards; each of them relatively longer than in female with the exception of genital segment. Second and 3rd segments lacking elytra, but strongly carinate on dorsal side to form broad, transverse, crescent plate. Fourth segment with carina much reduced and firmly coalesced with genital segment, border between them being indicated by whitish transverse band. Genital segment relatively much shorter and narrower than in female, conical, about as long as 2nd and 3rd segments combined together, and slightly decreasing in width backwards.

Legs are somewhat different in structure from those figured by Wilson[19] and by Ishii[9]. It appears certain variations to occur. Following is based on my specimens: First legs ovate, with 2 small closely adjoining lobes near base; basal one of these elongate triangular, tipped with 2 spines, while the other somewhat broader, without apical spine but fringed with a few minute hairs on distal border. Second legs also ovate, with short, linguiform, distinctly articulated ramus, tipped with 2 minute spines; margin just proximad to the ramus in the form of narrow quadrate lamella, at whose distal angle it bears 3 curved spines. Third legs round, neither with rudiment of ramus, nor with indentation. Genital segment bears at posterior end a pair of small foliaceous lateral papillae. It is problematical whether they represent 5th (or 6th) leg rudiments.

Genus *Lernanthropus* Blainville, 1822

Lernanthropus atrox Heller, 1865

(Figs. 3 & 4)

Lernanthropus atrox, Heller 1865, pp. 221–223, pl. 21, fig. 3; Heider 1879, pp. 80–81, pl. 4, figs. 59–61; Shishido 1898, pp. 216–218, 254–256, 6 figs.; Yamaguti 1936, pp. 16–17, pl. 9, figs. 98–105.

nec *Lernanthropus atrox*, Bassett-Smith 1898, pp. 91–92, pl. 6, fig. 3.

Occurrence: On *Sparus macrocephalus* (Basilewsky), at Momotori, Mie Pre-

fecture, by myself, June 28, 1951, 3 females and 4 males; on *Pagrosomus major* (T. & S.), at the same locality, same date, by myself, 1 male.

Distribution: Australian waters (HELLER) and Japan (SHISHIDO, YAMAGUTI).

Distribution in Japan: Tôkyô Bay (SHISHIDO) and Inland Sea (YAMAGUTI).

Hosts: *Pagrus guttulatus* (=*Pagrosomus unicolor*) [HELLER, YAMAGUTI], *Sparus macrocephalus* and *Pagrosomus major* [SHISHIDO]. Clinging to gills.

HELLER's[8] original, rather classic description was confined to that of the female and HEIDER's[7] subsequent one was based solely on the specimens HELLER had examined. No mention was made of the male up to SHISHIDO's[16] rediscovery of the species from Japanese waters. His specimens comprised both the sexes, but unfortunately, his description given in Japanese has not become universally known. YAMAGUTI's[22] subsequent account was rather short and not accompanied with figure of the male. Such being the case, it seems desirable to me to give a more precise description and figures on both the sexes, especially on their appendages based on my specimens anew.

Female: Color whitish yellow in preservatives, carapace and central part of thorax yellowish brown, becoming paler towards edges. Length from head to abdomen 3.62, from same to elytron 3.80, from same to 4th legs 4.96, carapace 1.32×1.64, thorax 2.68×1.78 (all in mm). Egg strings one and fifth times as long as body, eggs flattened, uniseriate.

Carapace expanded, wider than long, subquadrilateral, with trilobed front; antennal area slightly swollen, lentiform, and margin on either side of it somewhat more convex. Lateral margins irregularly undulated, making a right angle with anterior margin, and continuing back to strongly curved posterior margin. Thorax connected with carapace by a short narrowed neck, behind which it abruptly expands to form well-rounded shoulders, and gradually widens further backwards; lateral margins continue to those of 3rd legs without demarcation. Dorsal plate of 4th segment well developed, circular in outline, somewhat narrowing at base, only slightly wider than long, and longer than 2nd and 3rd segments. Genital segment small in size, barrel-shaped, concealed beneath dorsal plate. Abdomen further smaller, wider than long, somewhat narrowed backwards.

First antennae indistinctly jointed, with short setae at apex as well as on anterior border, and accompanying close to their bases a small round lamina, circumscribed by fine hairs, and an elongate acuminate lobe. Second antennae prehensile, connected with each other by a median strongly chitinized plate; terminal claw with well-curved apex and with a small mammiform papilla on posterior side near base. Mouth tube conical, widely apart from antennae. Preoral area occupied by 4 pairs of oval tubercles; 1st pair situated behind 2nd antennae, 2nd pair larger, close behind 1st pair, 3rd and 4th pairs just outside

Fig. 3. *Lernanthropus atrox* Heller.

A, female, ventral view; B, same, dorsal view; C, male, dorsal view; D–J, female; D, 1st antenna, ventral view; E, 2nd antenna; F, accessory spine of same; G, mouth tube, maxillae and protuberances on preoral area; H, 1st maxilliped; I, tip of same, further enlarged; J, 2nd maxilliped. A ×22, B ×18.5, C ×27, D, H ×120, E ×41, F, G, I ×340, J ×83.

the base of mouth tube and arranged side by side. Maxillae bifurcate; external ramus elongate, directed outwards and tipped with 2 spines, while internal ramus short, ovate, and with a single spine. First maxillipeds with proximal joint stout; distal joint slender, tipped by a curved claw, bearing a series of sharp denticles on inner margin. Second maxillipeds prehensile, feebler than 2nd antennae, with palm carrying minute basal spine; claw elongate, falciform, and with 2 denticles at about the middle of inner border. First thoracic legs locating on neck region, small-sized, and bifid; inner ramus triangular, with a short sharp apical spine, and finely ciliated on surface, outer ramus somewhat widening distally, truncate at end where 5 spines form a line. Basal joint has a short plumose spine just inside the inner ramus, and another similar but feebler one outside the outer ramus, and bears on its surface close to base of the latter a round lobe, circumscribed with fine hairs. Second legs situated internally to antero-lateral shoulders of thorax; outer ramus narrowing distally, with 4 apical spines, inner ramus somewhat turned outwards and with 2 blunt spines; basal joint strongly dilated, scattered with a few short hairs close to inner margin. Third legs lamellar, bilobed, present just in front of base of dorsal plate and on extreme lateral margin; 2 rami directed backwards, linguiform, outer one shorter but broader than the inner. Fourth legs bifurcate from the very base, both rami elongate ensiform lamellae, projecting backwards and extending far beyond posterior end of dorsal plate. (In the specimen shown in Fig. 3, A one of the rami of the right is entirely damaged.) Caudal rami invisible from above, being covered by dorsal plate, styliform, with swollen base and blunt apex, and bearing basal and 2 feebler apical hairs.

Male: Much smaller than female in size; from head to abdomen 2.08, from same to 4th legs 2.85, head 0.95×1.25, free segments 1.15×0.83 (all in mm). Color as in female, central part of carapace yellowish brown.

Carapace as that of female in shape, but more closely resembling a quadrangle, and with wider antennal area. Rest of body only slightly longer than carapace, a little wider than half of it and further narrower from bases of 3rd legs backwards. Genital segment fused with and indistinguishable from 4th segment which lacks dorsal elytron. Posterior end of body crescentic, with a pair of notch a little apart from base of abdomen. The latter short and small, cordiform, much wider than long.

Cephalic and thoracic appendages up to 1st legs as in female. Outer ramus of 2nd legs linguiform, with apex incurved, finely ciliated on surface, and surmounted with a sharp spine; inner ramus laminate, with insensibly bilobed end, each lobe tipped by a sharp, more or less curved spine and thickly setaceous on surface, or encircled by a ring of short setae. Third and 4th pairs of legs projecting from lateral sides of thorax, filiform, bifurcate from the very base; 2

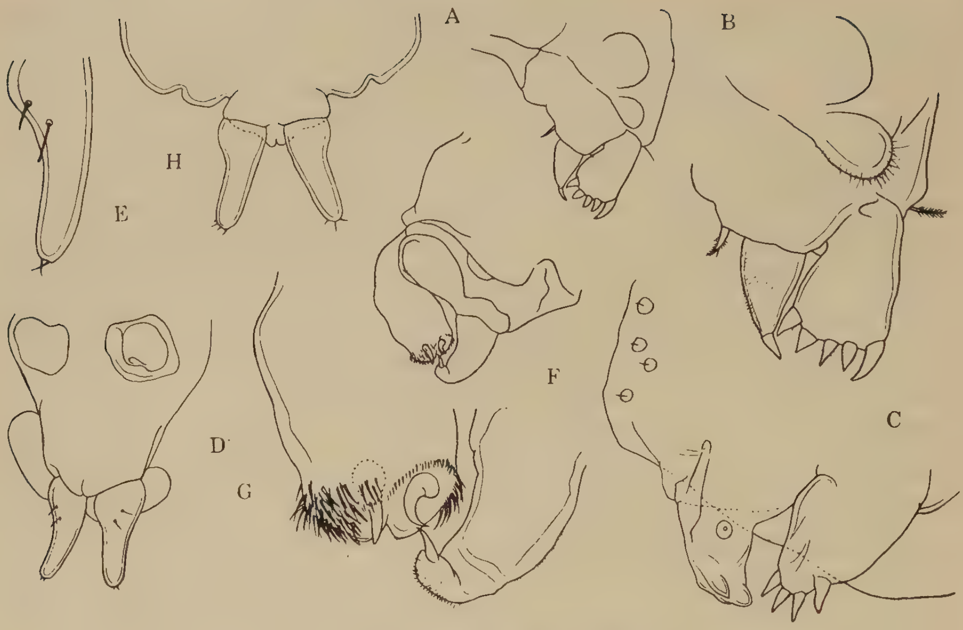

Fig. 4. *Lernanthropus atrox* Heller.

A–E, female; A, 1st swimming leg; B, rami of same, further enlarged; C, 2nd leg; D, genital segment and abdomen with caudal rami, ventral view; E, caudal ramus, further enlarged; F–H, male; F, 2nd leg; G, tip of same, further enlarged; H, genital segment, abdomen, and caudal rami, ventral view. A, C, E, H ×83, B ×170, D ×41, F ×120, G ×340.

rami subequal in length and shape in both pairs. Caudal rami with 3 apical spinules.

Remarks: Proportion of the carapace to the rest of body appears somewhat variable in the females from different sources. When we take the length of the former as 100, that of the latter (up to the end of elytron) is 118 in Heller's[8] figure, 162 in Heider's[7], 161 in Shishido's[16], and 175 in Yamaguti's[22], while 192 in mine.

My specimens are in accord with Shishido's almost in every detail in both the sexes except in the relative length of the female body parts named above.

In Heller's type[8] the 3rd legs have equal rami, but in the figure given by Heider (Fig. 59)[7] based on the specimens that Heller has examined the exopod is shorter, and in my specimens this is further shorter. The 1st antennae are, according to Heller, 2-segmented and the maxillae (his palp) 3-segmented. In my judgement, it appears that he recognizes on the antennae no more than the most proximal articulation, which is distinct also in my specimens, but he does not more distal, rather indistinct ones. Heider[7] says that "Die Fühler

des ersten Paares—zeigen eine Anzahl von Fältchen und Kerben, den Ueberresten einstiger Segmentgrenzen.—Eigentlich lassen sich aber nur zwei gesonderte Ringe an ihnen unterscheiden"; and in fact, generally speaking, articulation of antennae is inconspicuous in the present species. As regards the maxillae, there is a certain misleading in HELLER's observation. Since the 2 rami are closely adjoining together, it is probable that, being unable to find biramous nature of those limbs, he confuses the inner ramus with basal joint and considers the outer ramus as being 2-jointed. HELLER's description and figures on the other appendages are also inadequate from modern view point. It is striking, however, that the specimens from remote localities such as Australia and Japan are roughly in accord with each other in the constitution of appendages. I believe SHISHIDO was right in assigning his specimens to *atrox*.

On the other hand, YAMAGUTI's specimens are somewhat different from those of others in the outline of female carapace. But, as his specimens agree largely with mine in the structure of appendages, there may be hardly any doubt in the correctness of his identification.

BASSETT-SMITH (1898)[1] described a *Lernanthropus* from *Chrysophrys sarba* taken in the Persian gulf under the name of *L. atrox*. He gave no more than a brief description of the antennae on the female, but did more precise one on the male. On examination of his figures (Pl. 6, fig. 3, a–c) which accompany his description, his *atrox* does not conform with other specimens in some particulars. Although it has the very stout 3rd legs which are curved ventrally forming an arc, this is not the case with other specimens. Provided that the legs are fixed with preservative as they are turned ventrally from some cause, they may not attain so stout structures as figured by him. The 1st antennae, according to him, consist of 7 joints, 4th of these being the longest and issuing a knob-like process from the border. In ordinary *atrox* this pair of antennae show rather indistinct articulation and are not accompanied with such a process as he mentions. His male is also entirely different from that described above. The posterior part of the body is very large in size attaining more than twice the length of the carapace, whereas in my specimen it is rather short and narrow as compared with the carapace. The 4th legs projecting from about the middle of the thorax in his male are trailing from the posterior end in mine. Moreover, in his male the caudal rami are rather long and lancinate, the exopod of the 2nd legs has only 3 spines, and the endopod as well as the same of the preceding legs bear much longer spine than in mine.

On account of these differences, I am unable to agree with BASSETT-SMITH in referring his specimens to *atrox*.

Lernanthropus chrysophrys SHISHIDO, 1898

(Fig. 5)

Lernanthropus chrysophrys, Shishido 1898, pp. 337–340, figs. 1–4; Yamaguti 1936, pp. 15–16, pl. 9, figs. 92–97.

Occurrence: 2 females on gills of *Sparus macrocephalus* (Basilewsky) taken at Tomioka, Amakusa Is., November 2, 1950, by myself. No male.

Distribution: Endemic to Japan: Misaki (Shishido) and Inland Sea (Yamaguti).

Host: *S. macrocephalus* [Shishido] and *S. longispinis* [Yamaguti]. Hanging on gills.

As Shishido's original diagnosis was in Japanese, Yamaguti's is the sole one which foreign authors are available. Illustrations given by the latter, however, appear to be based on a somewhat abnormally fixed specimen. Unfortunately, the male has not been known.

Female: Color yellowish white in preservative. From front to abdomen 3.27, from same to dorsal plate 3.77, from same to 4th leg 5.14, head 0.96×1.64, thorax 2.86×1.73, anterior part of thorax 1.05×1.27, dorsal plate 1.77×1.75, egg strings 3.18 (all in mm).

Carapace evenly round in front on the median, produced on sides into large, oval, ventrally turned flaps and at postero-lateral angles into triangular, laterally extending lobes; posterior margin widely round. Anterior thoracic region quadrangular, constricted at frontal end to join carapace from which it is demarked by deep dorsal groove and by angular shoulders; lateral margins nearly parallel, with a slight depression just behind 2nd legs. Dorsal plate longer and wider than anterior region, circular in outline, convex dorsally, and completely concealing genital segment and abdomen. Genital segment close to anterior end of dorsal plate, small in size, nearly cordiform, narrowing backwards, with sides irregularly wrinkled. Abdomen shorter and narrower than preceding segment, triangular, broadening backwards; sides entire, posterior border somewhat arched and narrowly angular at lateral corners.

First antennae short, indistinctly 7-jointed, having setae at ends of apical 2 joints and on anterior border of basal 3 joints; they are accompanied close to base with 2-jointed filiform appendix. Second antennae prehensile, their bases connected across midline by a strongly chitinized plate; palm conical, attenuating towards end, with gracefully curved outer border and nearly straight inner border which bears near base a small triangular papilla, surmounted by a fine hair; terminal hook powerful, sharp, with strongly curved apex. Mouth tube conical, separated from 2nd antennae by a wide area, with 2 small tubercles arranged side by side just outside its base. Maxillae with outer ramus elongate, tipped

Fig. 5. *Lernanthropus chrysophrys* SHISHIDO, female.

A, female, dorsal view; B, same, ventral view; C, same, lateral view; D, 1st antenna; E, 2nd antenna; F, mouth tube with maxilla; G, apices of mandibles; H, 1st maxilliped; I, tip of same, further enlarged; J, 2nd maxilliped; K, 1st swimming leg; L, 2nd leg; M, genital segment and abdomen with caudal rami (4th legs removed), ventral view. A–C ×19.5, D, F ×170, E ×83, G ×48.6, H, M ×41, I, L ×340, J ×120, K ×206.

with 2 spinules and with inner ramus short, oval, bearing a single spinule. First maxillipeds with basal joint rather stout, succeeding joint much slender, with tiny sharp denticles on outer border near end, and tipped by short claw which is similarly denticulated along 2 borders. Just inside the bases of these maxillipeds and close to median line there are a pair of round pads which cover occasionally tip of mouth tube. Second maxillipeds feebler than 2nd antennae, similarly prehensile; basal joint relatively broad, with a low bulge close to base of inner border; claw strongly curved at apex, reaching the named bulge on palm. First legs situated at junction of thorax with carapace, biramous. Exopod quadrate, with 3 short triangular teeth on terminal border, while endopod shorter and narrower, conical, with apex carrying relatively longer spine and covered with minute hairs; basal joint with a short spine standing amidst small hairy area placed just outside the exopod. Second legs widely apart from each other, similar in structure to 1st, but exopod carrying 4 spines and endopod terminating in stouter spine and with hairs covering wider area; basal joint lacking outer spine and hairy area, and bearing a long spine surmounting on a round tubercle some distance apart from exopod. Third legs broad and long, foliaceous, emerging from posterior end of segment to which they belong and projecting ventrally in form of an arc; margins of each leg, further, curved in towards each other to make a deep groove on posterior side; apex well-rounded. Fourth legs attached to ventral face of dorsal plate near its anterior end and divided from near their bases into 2 equal rami which are elongate ensiform lamellae with pointed tip and extend backwards far beyond posterior margin of dorsal plate. Caudal rami relatively well developed, narrow, lancinate, pointing to the tip.

Male: Unknown.

Remarks: Yamaguti's figure on the female shows the 3rd legs as to direct laterally. It appears that the figure is based on a somewhat abnormally fixed specimen. In the character of other appendages his and my females are in strict accord with each other.

Bassett-Smith's *L. atrox* referred in the preceding section bears a certain resemblance to the present species. But until something more of his *atrox* is known, the propriety of placing it in the synonymy of *chrysophrys* is open to doubts.

Lernanthropus shishidoi new name

(Figs. 6 & 7)

Lernanthropus mugilii, Shishido 1898, pp. 120–126.
L. mugilis, Yamaguti 1936, pp. 13–15, pl. 7, fig. 79, pl. 8, figs. 80–88.
nec *L. mugilis*, Brian 1898, p. 216, pl. 3, fig. 17 (after Brian 1906).

Occurrence: 3 females, at Tomioka (Amakusa Is.), November 2, 1905, by myself; 17 females, and 3 males, at Tsu Fish-market (collected in the Ise Bay), December, 1952, by myself; 3 females, Ise Bay, by Mr. S. Kubota; 5 females and 2 males, Matusima, Miyagi Prefecture, October 5, 1952, by myself. All on gills of ***Mugil cephalus*** Linné.

Distribution: Japan. Rather common.

Host: *Mugil cephalus.* Clinging to gills.

YAMAGUTI (1923) in his description of this species suggested that SHISHIDO's name *mugilii* is "a lapsus calami or a typographical error and should be corrected to *mugilis*." Original description was published in Japanese in "Dobutsugaku-Zasshi" on April 15, 1898. In the same year, BRIAN described a *Lernanthropus* found on *Mugil auratus* from the Mediterranean under the same name, but the date of the issue is unknown to us.

YAMAGUTI pointed out an important difference between these forms, saying that BRIAN's species has a conspicuous conical appendage on each side of the thorax, "so that either of the two species should fall as a homonym." Although BRIAN's original work was inaccessible to me, his redescription appeared in 1906 conveys a good idea of the characteristics of his species. In addition to this, recently DELAMARE-DEBOUTTEVILLE & NUNES (1952) published excellent figures of BRIAN's species (Fig. 1). From these sources, I have become sure of the distinctness of the two forms in question.

The last named authors added that "l'une des deux espèces devra céder son nom.—Il faudra probablement trancher la question arbitrairement." In view of the fact that SHISHIDO's diagnosis given in Japanese was in all likelihood not known universally prior to YAMAGUTI's redescription, it appears to me desirable to change the name of Japanese species, which I propose to call *shishidoi* in honour of the discoverer of it.

Female: Red-colored in living state, but whitish in preservatives, from head to abdomen 4.75, from head to 4th legs 8.9, head 1.6/1.47, thorax (up to dorsal plates) 3.0×1.74 (all in mm).

Head 1/3 as long as body, nearly trapezoid, slightly narrowing anteriorly, with antennal area further narrowed, truncate on margin; posterior margin widely round. Thorax cylindrical, with anterior end narrower than and constricted from the rest by a pair of lateral incisions. Having somewhat undulated sides, it is widest in 3rd segment region and bears slight lateral notches just behind 3rd legs. Fourth segment carries at posterior end a pair of small linguiform dorsal plates which lie just outside of genital segment covering a part of these, but do not extend beyond abdomen. Genital segment small, transversely oblong ovate, and demarked from abdomen by cuneiform lateral sinuses. Abdomen with its anterior half resembling genital segment but slightly narrower;

posterior half flattened, narrowing backwards and ending in a V-shape. Caudal rami lateral, laminate, short and narrow.

Fig. 6. *Lernanthropus shishidoi* nom. nov., female.

A, female, dorsal view; B, another female, ventral view; C, 1st antenna; D, 2nd antenna; E, mouth tube and maxilla; F, tip of mandible; G, 1st maxilliped; H, outer border of apical joints of same, further enlarged; I, 2nd maxilliped; J, inner border of palm and claw, further enlarged; K, 1st swimming leg; L, 2nd leg; M, genital segment and abdomen, ventral view. A, B ×10, C, J ×170, D, I ×83, E, G, K, L ×120, F ×486, H ×340, M ×27.

First antennae distinctly 7-jointed, with apical joint very minute; this and next joints with short bristles, others sparingly or not setaceous. They accompany close to their bases 2 curved filiform accessory laminae, each surmounting on a tubercle. Second antennae prehensile; palm plump, with well-curved outer and nerly straight inner sides; claw short, hardly reaching centre of palm, with swollen base and curved apex. Mouth tube widely apart from antennae, conical. Outer ramus of maxillae elongate, rod-shaped, directed outwards, and tipped with 2 tiny spinules; inner ramus half as long as the outer, directed backwards, with 2 similar spinules. First maxillipeds stumpy; basal portion stout, with low fusiform bulge on posterior side, apical portion half as long as the basal, composed of 2 joints and unguiform flagellum, outer surface of which is circumscribed with a series of short but sharp denticles in a horse-shoe-shape; 3rd joint with similar denticles along outer border, these forming 2 linear rows arranged one behind the other. Second maxillipeds prehensile, somewhat feebler than 2nd antennae, palm ovate, bearing on inner border about the middle a finely serrated low bulge and very tiny tubercles from this bulge towards base; claw curved, sharp, with an acute denticle on inner border about the middle. First legs bipartite, exopod broad, with 5 strong spines on distal border, while endopod narrower, clavate, with angular tip and without spine; basal joint broad, with a feeble spine near base of endopod. Second legs with rami somewhat feebler than those of 1st, exopod with 4 short spines, endopod truncate, spineless, and basal joint with a spine near the base of exopod. Third legs short, undivided though slightly concave on distal margin, and not bent on themselves. Fourth legs elongate, bifurcated from the very base, 2 rami filiform, subequal, only a little shorter than body, and bluntly pointed; inner ramus trailing backwards, whereas outer ramus extending more or less laterally.

Male: Shorter than female, from head to abdomen 3.15, from same to 4th legs 5.40, head 1.37×1.37, thorax and abdomen 1.84×1.06 (all in mm).

Head rounded, with antennal area slightly produced forming a trapezium. Thorax as long as head, but narrower, with 2 pairs of lateral notches, one just behind head and the other in front of 3rd legs. No dorsal plate. Genital segment small, roughly triangular, narrowing backwards and irregular on margins. Abdomen further narrower, claviform, ending in a V-shape. Caudal rami as in female.

Second legs with exopod broad, roughly ovate, and constricted at base, distal border indistinctly divided into 2 laminae, finely setaceous and with 4 irregularly disposed spines; endopod conical, with blunt apex, and covered by minute setae at apex as well as on outside near base; basal joint spineless. Third legs uniramous, filiform, as long as free segments exclusive of abdomen, and projecting laterally. Fourth legs biramous, with 2 rami subequal, about

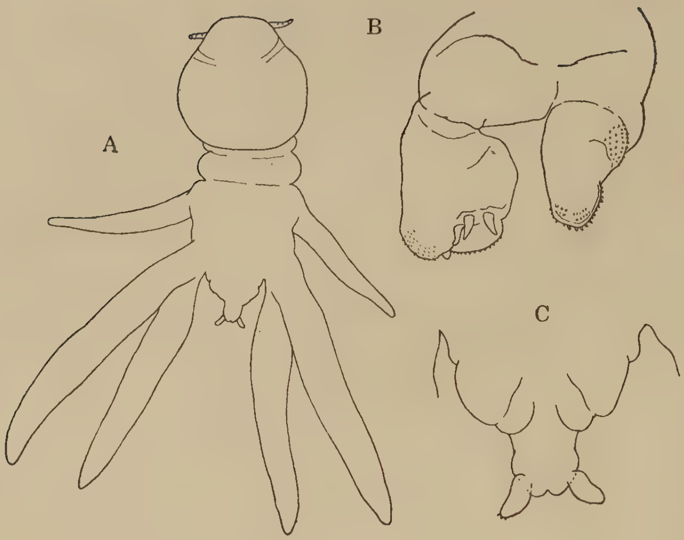

Fig. 7. *Lernanthropus shishidoi* nom. nov., male.
A, male, dorsal view; B, 2nd leg; C, genital segment and abdomen, ventral view. A ×12.7, B ×170, C ×42.

twice as long as preceding legs, and extending postero-laterally. Otherwise as in female.

Remarks: The new species may be distinguished from *mugilis* BRIAN[3,5] by the characters tabulated below:

	mugilis BRIAN	*shishidoi* nom. nov.
Head	broader forwards, with lateral processes	broader backwards, without lateral processes
4th thoracic segment	without dorsal plates	with paired dorsal plates
3rd legs	large, foliaceous, shallowly but distinctly bifurcated, and curved over themselves	small, not bifurcated, and not bent on themselves
4th legs	about as long as thoracic segments	about one and half times as long as thoracic segments

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Copepods Parasitic on Japanese Fishes

9. Family Chondracanthidae, Subfamily Chondracanthinae*

(*With 24 Text-figures*)

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So far as I am aware, 16 species of the Chondracanthinae have been known to occur in Japanese waters. The present paper dealing with this subfamily comprises the descriptions of 1 new species of *Chondracanthus* and 8 new ones of *Acanthochondria* and supplementary notes for 3 previously known ones belonging to the former genus.

As is usual in this subfamily, all of the species here dealt with are the parasites of bottom fishes clinging to gill filament, to gill arch, or to the wall of buccal cavity. Some of them bury the anterior part of the body in the host flesh, which closely surrounds it forming a swelling on the surface of epithelium. Such a mode of living is one of the characteristics of the Lernentominae, another subdivision of the Chondracanthidae. In this subfamily, however, the mouth parts are far removed from the antennae to situate close to the thoracic legs owing to the strong prolongation of head which bears, in addition, a number of processes for anchoring the body within the host flesh. All the species described below, even those burying their head in the host tissue, never show such an aberrant character.

Some confusion and misleading existed in the descriptions of the Chondracanthidae regarding terminology of certain segments and appendages. Usage of the terms, however, has been corrected and made more rational by Heegaard (1947) through the results from his extensive studies on the ontogeny of parasitic copepods. The greatly inflated segment which occupies a larger part of the female body was formerly called by various names, for example, trunk segment, genital segment and so on, and the small-sized segment at the posterior extremity was termed simply the abdomen. According to that author, the inflated segment is a composite one resulted from the union of the 3rd and 4th thoracic segments and the so-called abdomen is made up of much reduced genital segment and similarly reduced true abdomen.

As concerns the abdomen in older sense, some authors speak of it as

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1-segmented and others as 2-segmented. The view of the former appears, with all probabilities, to have originated in an inaccurate examination. Being always very small in size, this part of the body is partly overlapped by paired posterior processes of the 4th segment from above and by the bases of ovisacs from below, so that it is occasionally rather difficult to make out the true structure. In all of my specimens, a clear articulation is observed between the two component segments.

Most of the authors interpret the laminate appendage present just behind the mandible as its exopodite, or its palp. HEEGAARD is of the opinion that this represents the 2nd maxilla. Maxilla of WILSON[22], or the 2nd maxilla of OAKLEY[14] becomes, accordingly, the 1st maxilliped, and its end segment is said to correspond to the exopodite whereas styliform appendix to the endopodite. The last oral appendage which has been called simply maxilliped is the 2nd maxilliped. In the descriptions given below, HEEGAARD's designation will be followed except for calling the 2nd maxilla simply the maxilla, as the 1st one is missing in this family.

Caudal ramus of the female overlooked by the majority of authors has been discovered by HEEGAARD and this is always present in all the species dealt with below. Usually, this is displaced from the posterior end of the abdomen to the ventral side more or less close to its junction with genital segment.

Sometimes, though not always, a pair of minute unguiform appendices which have not been alluded by any of authors are found on the ventral surface of genital segment. Although nothing is known about the true nature of them, it appears to me probable that they represent the remnants of appendages.

Male and occasionally female also have the body more or less strongly curved ventrally. Measurements indicated in the following descriptions are taken as the body is curved and are represented by mm.

All the type specimens of the new species to be proposed in this report will be lodged in the Prefectural University of Mie.

Genus *Chondracanthus* DE LA ROCHE, 1811

Chondracanthus distortus WILSON, 1922

(Figs. 1 & 2)

Chondracanthus distortus, WILSON 1922, pp. 8–11, pl. 4, figs. 33–39; YAMAGUTI 1939, pp. 531–532, pl. 34, fig. 8; HEEGAARD 1945, p. 25.

Occurrence: Attached to gills, to gill arches or to deeper part of buccal cavity of *Zenopsis nebulosa* (T. & S.); numerous specimens collected at Owase, Mie Prefecture, September 23, 1951, December 8, 1952, and November 19, 1953, by myself.

Distribution: Endemic to Japan; Sato-no-misaki, Kyûshû; Osesaki, Gotô Is. (Wilson); Suruga Bay (Yamaguti), Kagosima (Heegaard).

Hosts: *Zeus japonicus* [Wilson], *Z. faber* [Heegaard], and *Zenopsis nebulosa* [Yamaguti].

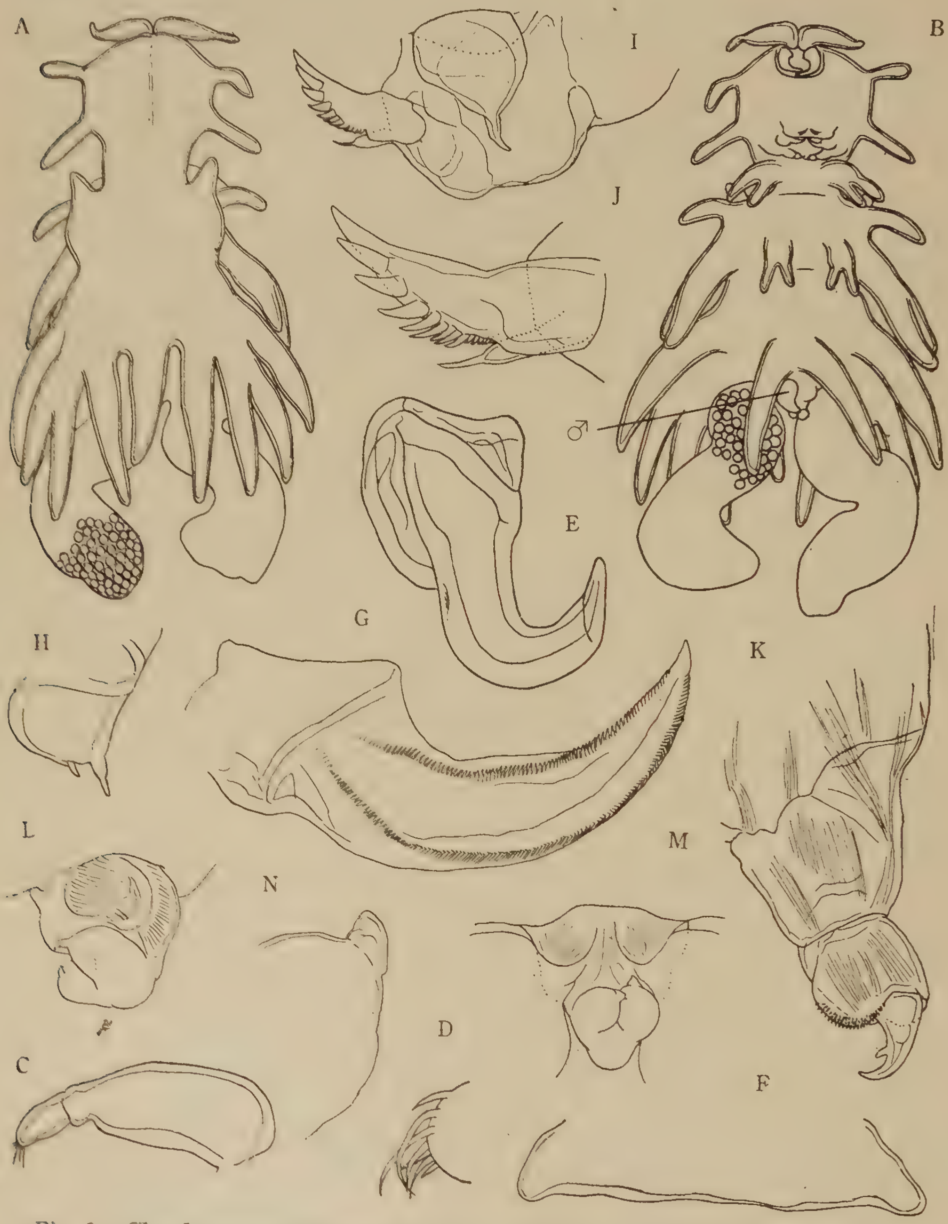

Fig. 1. *Chondracanthus distortus* Wilson, female. A, dorsal view; B, lateral view; C, 1st antenna; D, apex of same; E, 2nd antenna; F, labrum; G, mandible; H, maxilla; I, maxilla and 1st maxilliped in situ; J, 2 rami of 1st maxilliped; K, 2nd maxilliped; L, genital segment and abdomen, lateral view; M, same, ventral view; N, caudal ramus. A, B ×12, C, L, M ×41, D, H, I, K, N ×170, E ×83, F ×120, G, J ×340.

Despite WILSON's[21] fairly precise description of this species, I feel it necessary to make some additional remarks.

Female: Uniformly whitish, with egg strings yellowish. Length from head to abdomen 4.00, same to longest posterior process of thorax 5.22, same from antennae to egg tubes 6.49, head 1.17×1.44, width across cephalic barbs 2.44, length of thorax and abdomen 2.78, width across thoracic processes 3.56, same in 2nd free segment exclusive of processes 1.78.

WILSON's statement on the arrangement of thoracic processes is somewhat inadequate. First thoracic segment without process. Second segment with 3 pairs of lateral processes projecting from antero-dorsal, antero-ventral and postero-ventral corners respectively; 1st of these short, directing forward, 2nd slightly longer, projecting laterally, and 3rd again longer, bifurcate, trailing diagonally backwards. Third and 4th segments confluent, armed with 4 processes locating dorsally to egg tube on each side and extending from caudal end further backwards; incision between central 2 of them somewhat shallower than that between others. Processes of 2 sides interpose a single median one on dorsal side and paired ones on ventral side which are close to either side of mid-line. Genital segment globular, small in size. Abdomen similar in shape, but further smaller, and furnished with relatively large-sized bulbous caudal rami, apical spine of which is replaced by a short conical papilla.

First antennae biarticulated, tipped with a few feeble hairs as stated by YAMAGUTI[23]; line of articulation locates, in some cases, more proximally than indicated in Fig. 1, C, dividing antenna into 2 subequal joints. Second antennae lacking accessory spines alluded by WILSON. Labrum without any tubercles along free margin. Teeth fringing mandibular blade finer, more closely set, and much larger in number than in ordinary case. First maxillae with 1 or 2 spines; when 2 are present, 1 of them is retarded. Claw of 2nd maxillipeds with spiniform process instead of spine; lunular expansion of succeeding joint with marginal rows of fine spinules. In other points as in WILSON's description.

Male: Length 0.43, head, length on dorsal side 0.33, width 0.23, thickness 0.25, free segments, length on dorsal side 0.27, width 0.14, thickness 0.17. Color whitish, translucent.

First free segment undifferentiated from cephalothorax. Succeeding segments also indistinct from one another, 4 poorly-developed lines of articulation remaining only on dorsal side. First antennae unjointed, sausage-shaped, apically setaceous. Second antennae provided on outside of their bases with a minute knob-like appendix, tipped with 2 spinules. Labrum destitute of tubercles. Marginal teeth of mandibles triangular. Maxillae ovate, with 2 unequal spinules. Exopod of 1st maxillipeds slightly curved, toothed on distal half of posterior margin; endopod styliform, regularly acuminating to tip. Claw of 2nd maxillipeds

with only slightly curved extremity and without accessory process; inner border of penultimate joint has sparse row of fringing spinules which extends as far as to outer corner of claw base. Two pairs of rudimentary legs equal in size and in structure. They are unjointed triangular laminae carrying sharp spinule at tip as well as at about the middle. Caudal rami conical, ending in sharp apex, with a short spiniform accessory branch at about the middle and 2 basal hairs, arranged one on dorsal and the other on ventral sides.

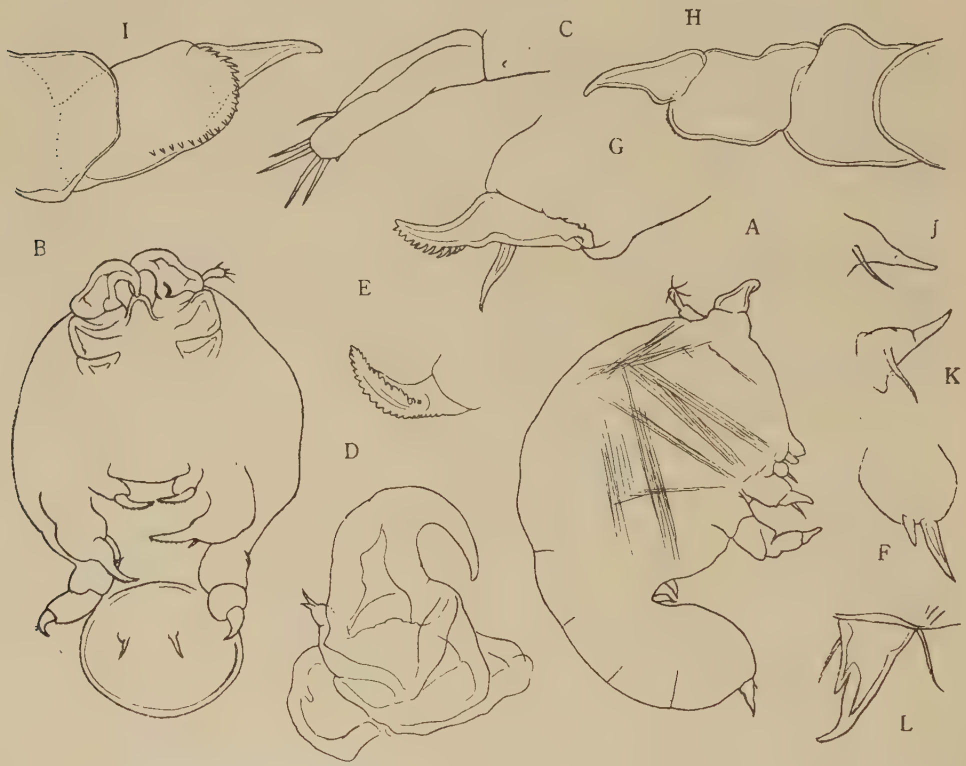

Fig. 2. *Chondracanthus distortus* WILSON, male. A, lateral view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, mandible; F, maxilla; G, 1st maxilliped; H, 2nd maxilliped, outer view; I, same, inner view; J, 1st leg; K, 2nd leg; L, caudal ramus. A ×120, B ×170, C, D, H ×486, E–G, I–L ×593.

Chondracanthus theragrae YAMAGUTI, 1939

(Figs. 3 & 4)

Chondracanthus theragrae, YAMAGUTI 1939, p. 531, pl. 34, figs. 1–7.

Occurrence: On gill arch of *Doederleinia berycoides* (HILGENDORF); a single female accompanied by a male taken at Owase, Mie Prefecture, December 9, 1952, by myself.

Distribution: Toyama Bay (Japan Sea); on *Theragra chalcogramma* [YAMAGUTI].

To YAMAGUTI's[23)] detailed description I have only to add a few notes.

Female: Length from head to abdomen 4.4, same to posterior processes

Fig. 3. *Chondracanthus theragrae* YAMAGUTI, female. A, dorsal view; B, ventral view; C, head, dorsal view; D, 1st antenna; E, 2nd antenna; F, mandible, maxilla, 1st and 2nd maxillipeds, and labrum in situ; G, 1st leg; H, 2nd leg; I, genital segment and abdomen; J, caudal ramus. A, B ×10, C ×18.7, D ×41, E, G–I ×83, F ×170, J ×194.

of thorax 4.72, same to egg tubes 6.6, head 1.8×2.93, width across barbs 3.33, length of thorax including posterior processes 4.0, width excluding lateral processes 1.67, same across anterior processes 3.18, egg tubes, length 6.6. Body and egg strings whitish in preservative.

Anterior end of body curved ventrally in the present specimen. Thorax forms a short narrowed neck to join head. Labral margin depressed on the median, without tuberculation. Maxillae bear a single spine. Exopod of 1st maxillipeds toothed only near apex. First legs somewhat stouter than 2nd; inner branch of both pairs a little narrower than outer branch which has a short spine on outside close to base. Caudal rami with a stout curved apical and a feeble outer basal spines.

Male: Length 0.48, head, length 0.29, thickness 0.28, free segments, length along dorsal side 0.36, same, along ventral side 0.17, thickness 0.17. Whitish, pellucid.

Free segments thick in proportion to head, scarcely with any trace of articulation. Frontal plate semicircular. First antennae 2-jointed; 1st joint broadening basally, with a single subterminal hair on anterior side, 2nd joint cylindrical, longer, and tipped with a few hairs. Second antennae somewhat plump. Labrum non-tuberculate. Teeth of mandibles rather sparse. Maxillae

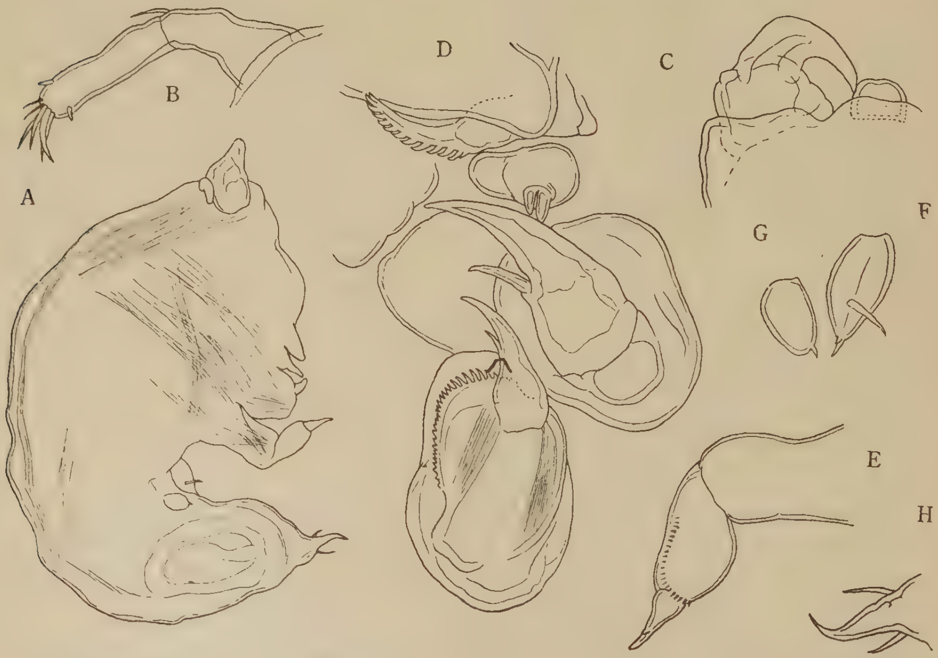

Fig. 4. *Chondracanthus theragrae* Yamaguti, male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, labrum, mandible, maxilla, 1st and 2nd maxillipeds in situ; E, maxilliped, inner view; F, 1st leg; G, 2nd leg; H, caudal ramus. A ×120, B, D ×486, C, E–H ×340.

with 2 unequal bluntly pointed spines. Exopod of 1st maxillipeds slightly curved, acuminating towards tip, not toothed; endopod simple, styliform. Claw of 2nd maxillipeds resembling exopod of preceding limbs, but feebler, with spiniform accessory process; penultimate joint with inner row of fine spinules ranging from outer distal angle of joint as far near as base. Two pairs of leg rudiments oblong ovate. Anterior pair longer than posterior, furnished with 2 minute spinules of unequal sizes at apex and a single far longer one at about the middle. Posterior pair unarmed except for having a single minute apical spinule. Caudal rami conical, sharply pointed, and carrying 2 basal setae, one on dorsal and the other on ventral sides.

Chondracanthus polymixiae YAMAGUTI, 1939

(Figs. 5 & 6)

Chondracanthus polymixiae. YAMAGUTI 1939, pp. 532–533, pl. 35, figs. 9–16.

Occurrence: On *Polymixia japonica* GÜNTHER, at Owase, Mie Prefecture, December 8, 1952, by myself. 4 females each associated with a male, clinging to floor of buccal cavity of host close to line of attachment of last gill arch.

Distribution: Suruga Bay (Pacific side of Japan); on inner face of operculum of same host (YAMAGUTI).

Following is an additional note to YAMAGUTI's detailed description[23].

Female: Length from head to abdomen 4.72, same to posterior processes of thorax 5.56, same from antennae to egg cases 7.68, head 1.39 / 1.78, width at 1st free segment 1.17, same at 2nd segment 1.61, same including lateral processes 2.56, egg cases, length 3.06. Body and egg cases whitish.

Head hemispherical in dorsal aspect, cuneiform in lateral aspect, with an elliptical dorsal swelling close to posterior border from which it decreases in thickness forwards; postero-lateral processes blunt. First thoracic segment in form of a narrowed neck connecting head with succeeding segment, and with prominent dorsal bulge, but no lateral process. Second segment with a similar bulge and distinct shoulders. Bulge on 3rd and 4th segments somewhat lower than that on previous segments and present close to posterior border of each segment; lateral margin wrinkled, without developing shoulders. Lateral processes directing backwards, those of last segment turning slightly inwards in addition.

First antennae lamellar, with a few minute hairs on posterior margin. Mandibles sharp at apex and thickly dentate on 2 borders; denticles undeveloped for some distance from apex on inner border and for a shorter distance on outer border. Maxillae with 2 unequal apical spines. Exopodite of 1st maxillipeds has a solitary subterminal tooth on the border opposite to that which bears series of teeth; endopod rod-shaped, terminating in a spine. Claw of 2nd maxillipeds

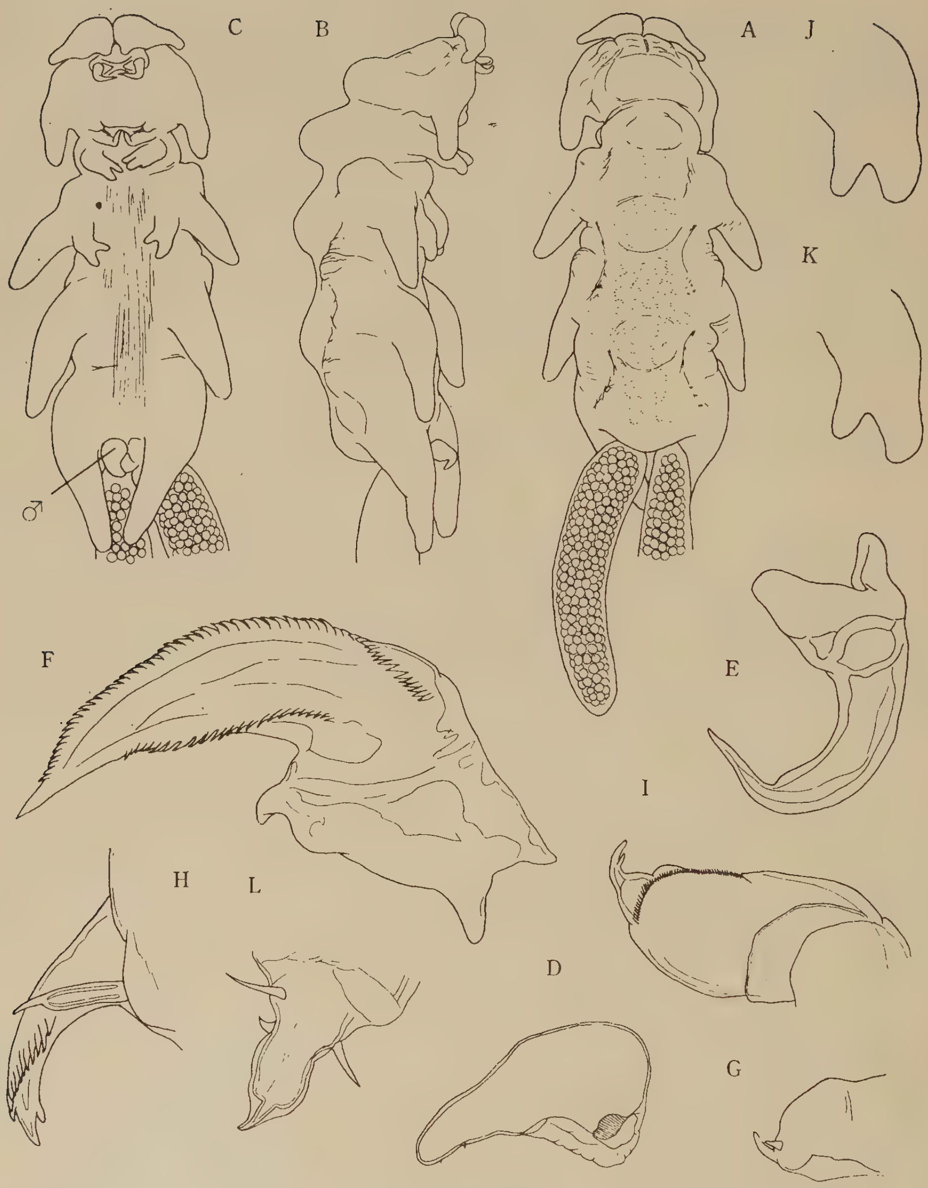

Fig. 5. *Chondracanthus polymixiae* YAMAGUTI, female. A, dorsal view; B, lateral view; C, ventral view; D, 1st antenna; E, 2nd antenna; F, mandible; G, maxilla; H, 2 rami of 1st maxilliped; I, 2nd maxilliped; J, 1st leg; K, 2nd leg; L, caudal ramus, lateral view. A–C ×12, D, J, K ×41, E ×83, F, H ×340, G, I, L ×170.

feeble, swollen at base, strongly narrowed at apex where it carries a short inner spiniform branch; penultimate joint with crescentic row of spinules ranging from outer distal angle to center of inner border. Caudal rami with 1 terminal and 3 basal spines, the latter disposed 1 on dorsal and others on ventral sides.

Male: Length 0.52, head, length on dorsal side 0.32, thickness 0.24, free segments, length on ventral side 0.17, thickness 0.13. Colorless, translucent.

Head oblong, insensibly continuing to thorax without any demarcation. Thoracic segmentation indistinct. Frontal plate trapezoid. First antennae unjointed, columnar, having setae arranged on anterior margin at regular intervals and a solitary one on posterior margin behind apex which bears a bunch of a few setae. Second antennae broad at base, with a round external bulge, but

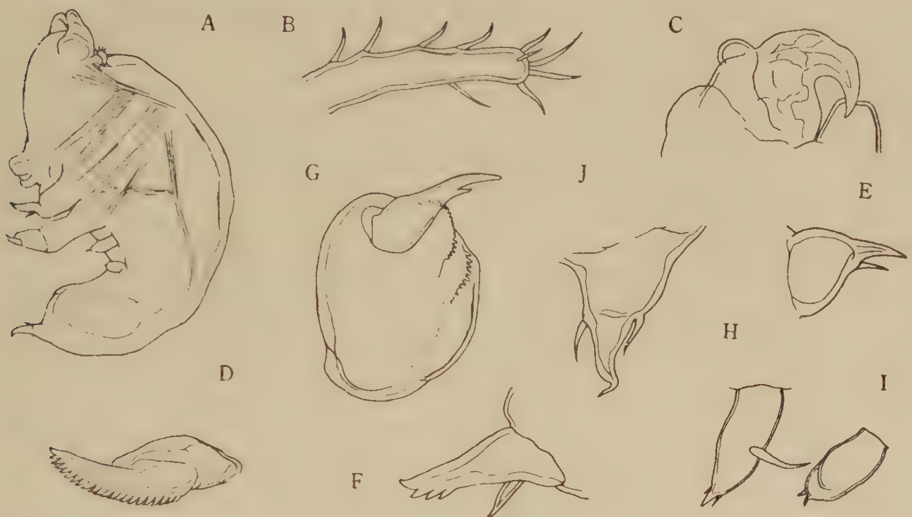

Fig. 6. *Chondracanthus polymixiae* YAMAGUTI, male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, mandible; E, maxilla; F, 1st maxilliped, apical part; G, 2nd maxilliped, apical 2 joints; H, 1st leg; I, 2nd leg; J, caudal ramus, lateral view. A ×83, B, D–G ×486, C, H–J ×340.

sharp and beak-like at apex. Mandibular blade regularly dentate. Maxillae with 2 unequal sharp spines. Exopodite of 1st maxillipeds acuminating, armed with only 3 marginal teeth, endopod styliform. Claw of 2nd maxillipeds relatively longer than that of female, slender, with short spiniform accessory branch; teeth on inner border of penultimate joint making 2 crescentic rows arranged in front and in the rear. First pair of legs elongate, acute at tip, and carrying a stout spine about the middle and a short one at tip which is associated with feebler one on each side. Second pair shorter than 1st, having round apex surmounted with 2 minute spinules and a bulge a little behind. Caudal rami conical, rather broad at base, sharp at apex, obscurely biarticulated, and with 2 spines at the articulation, 1 on each of dorsal and ventral sides.

Chondracanthus gibbosus n. sp.

(Figs. 7 & 8)

Occurrence: On gill arch of *Zenion japonicum* KAMOHARA, 100–200 fathoms

off Minabe, Wakayama Prefecture, by Mr. S. Sakaguti; a single couple. Female buries its head in soft tissue of under surface of gill arch, trailing its body between gill filaments; male attached to abdomen of female.

Female: Holotype. Body stumpy, crooked, rather short in relation to its width, and thick dorso-ventrally. Length from head to abdomen 2.00, same to dorso-median process of thorax 3.00, same to egg tubes 3.64, head 0.71×1.07, depth 0.79, thorax, length up to dorso-median process 2.54, width across widest region excluding processes 1.64, same including processes 2.61, egg tubes, length 1.71. Color brownish yellow in preservative, with yellowish egg tubes.

Head distinct from free segments, wider than long, cordiform, having round sides and somewhat protruding front; dorsal surface nearly flat, ventral greatly swollen. First 2 thoracic segments crowded together, demarcation between them indicated merely by a short mid-ventral surface groove. The composite segment has an oval dorso-median bulge and a pair of lateral processes which are elongate, cylindrical, blunt and carry a short papilliform branch on dorsal side close to base. Third segment demarked from 2nd by shallow groove both dorsally and ventrally, but from 4th only by ventral groove; dorsal surface raised in a broad transverse ridge. Third segment provided with an elongate, blunt, postero-laterally directing process at each ventro-lateral corner and with a short clavate one dorsally to its base and a little in front. Fourth segment armed with 7 elongate processes around its posterior margin, these being arranged 1 on mid-dorsal line and 3 on each side extending further backwards. Median process with broad, somewhat swollen base, but abruptly narrowed at about 1/3 of its length, then tapering. Lateral processes disposed dorso-ventrally and increasingly longer from dorsal to ventral. Dorsal member of them located just outside median process, interposing triangular sinus, and reinforced with a short outer accessory branch near base; ventral member on each side of median line, simply lanceolate, with blunt apices, and resembling the rest which is central. Genital segment rather small in size, broader than long, hemispherical, inserted between ventral processes of preceding segment by which lateral regions are overlapped. Abdomen further smaller, transversely oval, unsegmented.

First antennae triangular unjointed lamellae and bearing a few tiny hairs at narrowed end. Second antennae hook-like, with apical part sharp and making a right angle with basal part. Labrum concave on margin, without tuberculation. Mandibles falciform, with bordering denticles somewhat feebly developed. Maxillae campanulate, with 2 unequal apical spines. Exopod of 1st maxillipeds not much curved, with a sharp tip and a few teeth on terminal half of posterior border; endopod short, spiniform, ending in a point. Claw of 2nd maxillipeds acuminate towards tip, without accessory branch, 2nd joint with distal bulge rather feebly spinulose. Two pairs of legs arranged almost side by side rather than antero-

posteriorly; 1st pair just outside and a little in front of 2nd pair which are close to mid-ventral line. They are subequal in size, laminate, and have rounded proximo-lateral angles and bifid ends, 2 branches remaining short and blunt.

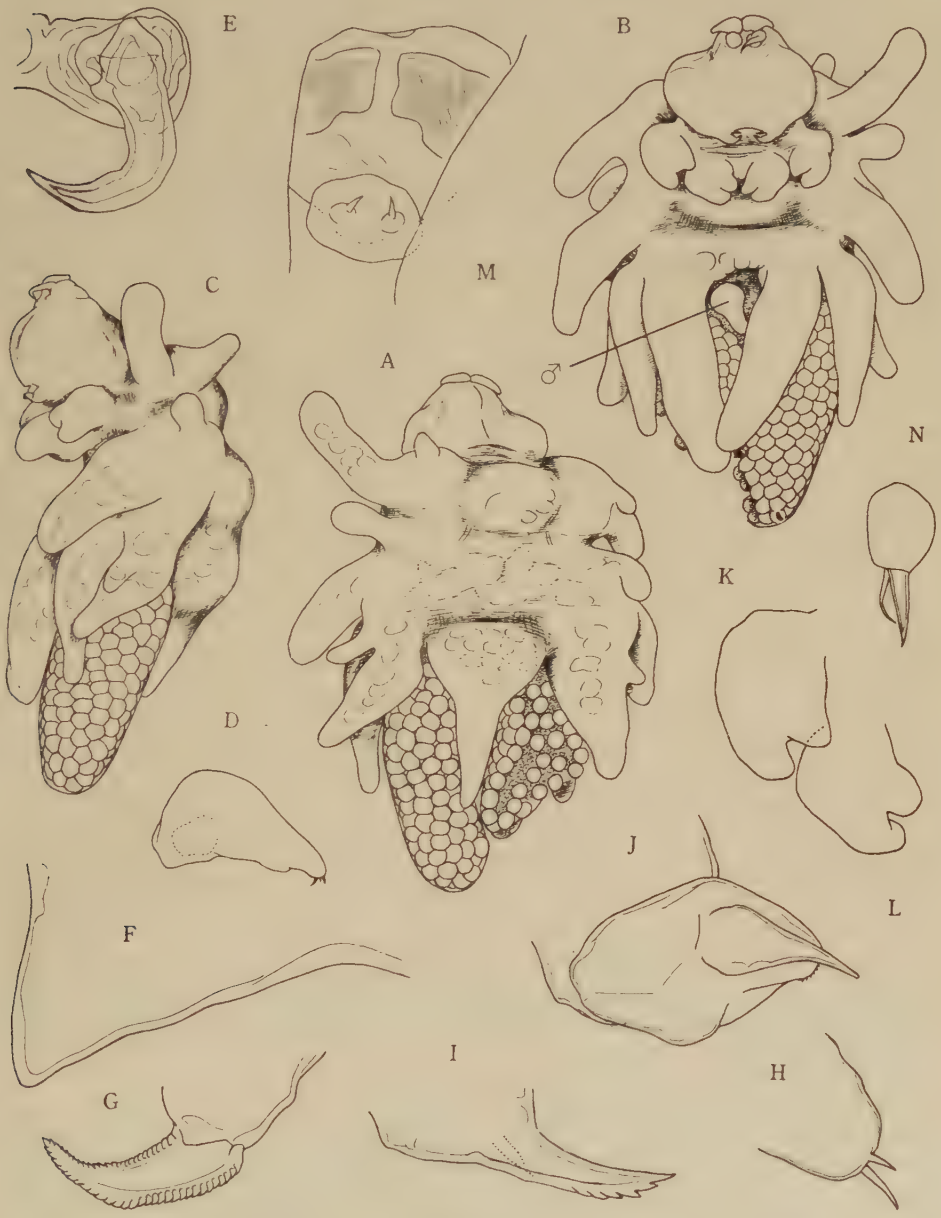

Fig. 7. *Chondracanthus gibbosus* n. sp., female. A, dorsal view; B, ventral view; C, lateral view; D, 1st antenna; E, 2nd antenna; F, labrum; G, mandible; H, maxilla; I, 1st maxilliped; J, 2nd maxilliped; K, 1st leg; L, 2nd leg; M, posterior part of body showing genital segment and abdomen; N, caudal ramus. A–C ×18.7, D, E ×120, F–J, N ×480, K, L ×52, M ×83.

Caudal rami bulbous, tipped with sharp stout spine and feeble hair. Egg tubes short in relation to their thickness, sausage-shaped; eggs pluristichous.

Male: Dwarf, colorless, translucent. Length 0.42, head, length along ventral line 0.42, same along dorsal line 0.25, thickness 0.20, free segments, length along ventral line 0.17, same along dorsal line 0.26, thickness 0.13.

Head highly convex on dorsal face, nearly flat on preoral ventral face. First thoracic segment obscurely discriminated from head by a faint transverse line. Segmentation of rest of body largely disappeared, retaining only 2 short lines of boundaries on lateral side. Frontal lamina triangular, with blunt apex. First antennae filiform, indistinctly 2-jointed, with apical bunch of hairs and a few shorter ones scattered on anterior border. Second antennae have broad base with small external bulges; apex sharp, recurved backwards. Mandibles fringed

Fig. 8. *Chondracanthus gibbosus* n. sp., male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, mandible; E, maxilla; F, 1st maxilliped; G, 2nd maxilliped; H, apical part of same, from inner side; I, 1st leg; J, 2nd leg; K, caudal ramus. A ×120, B–K ×486.

with triangular teeth. Maxillae with 2 equal spines. Exopod of 1st maxillipeds only slightly curved, with sharp extremity, and entire on margin; endopod with spiniform abruptly narrowed apex. Claw of 2nd maxillipeds curved only at its narrowed end, deprived of accessory process; penultimate joint with distal bulge and with rows of tiny spinules along inner border ranging nearly from base to outer distal angle of bulge. Two pairs of leg rudiments represented by relatively well-developed, oblong ovate, jointed laminae; boundary of joints indicated by

incomplete line traversing laminae distally to their centre. Proximal joint with a long spine a little behind the end in both pairs, distal joint with 2 minute terminal spinules in 1st legs, while with 1 in 2nd. Caudal rami conical, with pointed somewhat crinkled tip and bearing 3 hairs at about the middle, 1 on dorsal side and the rest on ventral side.

Remarks: In that the thorax carries a number of processes arranged in a bouquet-like manner, this species is similar to *distortus* WILSON[8,21,23], *neali* LEIGH-SHARPE[11] and *zei* DE LA ROCHE[2,3,14,17,19,23]. It differs from these not only in the number of processes, but also in the absence of cephalic barbs. Moreover, egg tubes of this species are not tortuous as in the first 2 of the named species.

Genus *Acanthochondria* OAKLEY, 1927

Acanthochondria incisa n. sp.

(Figs. 9 & 10)

Occurrence: On *Heliocolenus dactylopterus* (DE LA ROCHE) taken at Owase, Mie Prefecture, December 8, 1952, by myself. Female buries its head and 1st thoracic segment in flesh of roof or floor of buccal cavity, or of gill arch. A female is selected as holotype out of numerous specimens.

Female: Holotype. Length from head to egg tubes 6.8, same to abdomen 4.09, same to thoracic processes 3.39, head 1.25 × 1.08, its thickness 1.05, length of thorax exclusive of processes 3.16, 1st thoracic segment 0.40 × 0.99, 2nd segment 0.66 × 1.58, 3rd plus 4th segment, length 2.11, 3rd segment, width 2.37, 4th segment, same 2.17, egg tubes 3.03 long. Color white, egg tubes yellowish.

Body short in relation to width, a little curved ventrally. Head globular, with convex dorsal and flat ventral surfaces. First thoracic segment in form of a short collar, narrower than and distinct from either of head and 2nd segment. Second segment with dorsal face flattened to form transversely oblong plate with round sides, longer than 1st segment and continuing to 3rd through narrow articulation. Third and 4th segments consolidate, boundary being indicated merely by deep V-shaped lateral notches present about the middle, in front and in the rear of which both sides are well-rounded. Posterior end of 4th segment produced on eigher side in a short triangular process, whose apex is blunt and turned inwards. Genital segment small, hemispherical, bearing at end a short, blunt, ventrally curved abdomen.

First antennae filiform, 2-jointed, slightly geniculate at junction of joints, non-hairy; proximal joint occupying greater part of length. Second antennae hook-like as usual, with apical part slender and sharp. Labrum tuberculate on free margin. Mandibles robust, falcate, coarsely dentate along borders. Maxillae helmet-shaped, with stout knotty spine at tip and feebler curved one slightly

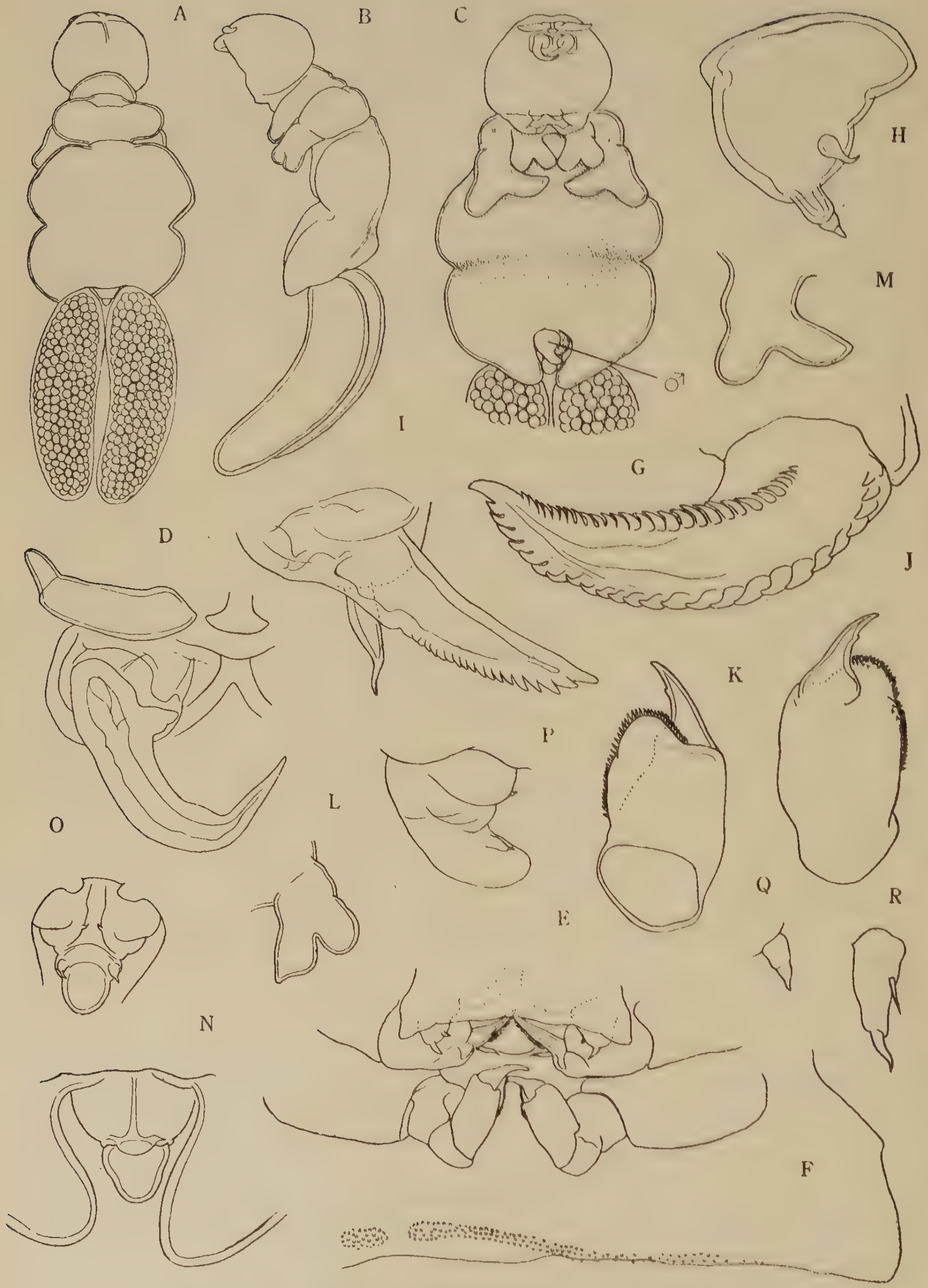

Fig. 9. *Acanthochondria incisa* n. sp., female. A, dorsal view; B, lateral view; C, ventral view; D, 1st and 2nd antennae in situ; E, oral region with appendages in situ; F, labrum; G, mandible; H, maxilla; I, apical part of 1st maxilliped; J, apical part of 2nd maxilliped, posterior view; K, same, anterior view; L, 1st leg; M, 2nd leg; N, genital segment and abdomen, dorsal view; O, same, ventral view; P, same, lateral view; Q, limb rudiment on genital segment; R, caudal ramus. A, B ×10, C ×15.3, D, E ×83, F–I ×348, J, K, Q, R ×170, L, M ×27, N P ×41.

behind. Exopod of 1st maxillipeds acute towards tip, almost straight, dentate on posterior border; endopod rod-shaped, ending in spine. Claw of 2nd maxillipeds with broad base and acuminate tip, insensibly curved, and with a tubercle on inner border representing remnant of accessory process; inner distal angle of penultimate joint dilated to form a semicircular lobe, which together with inner border of joint is marginated by a row of spinules. First pair of legs smaller than and internal to 2nd. Both pairs have outer ramus blunt and inner ramus more or less pointed at inner distal angle; rami of 2nd pair more divergent than in 1st. Genital segment with a pair of unguiform squama on ventral side, 1 on each side of midline. Caudal rami conical, with a spine at end and a hair about the middle. Egg tubes sausage-shaped, slightly curved ventrally.

Male : Pygmy, strongly crooked, colorless and pellucid. Length from antennae to posterior end 0.48, same, exclusive of antennae 0.42, head, length on dorsal side 0.32, thickness 0.28, width 0.29, free segments, length on dorsal line 0.23, width 0.16, thickness 0.11.

Head globular in dorsal aspect, ovate in lateral aspect, less convex ventrally than dorsally. First thoracic segment inseparable from head. Border lines bet-

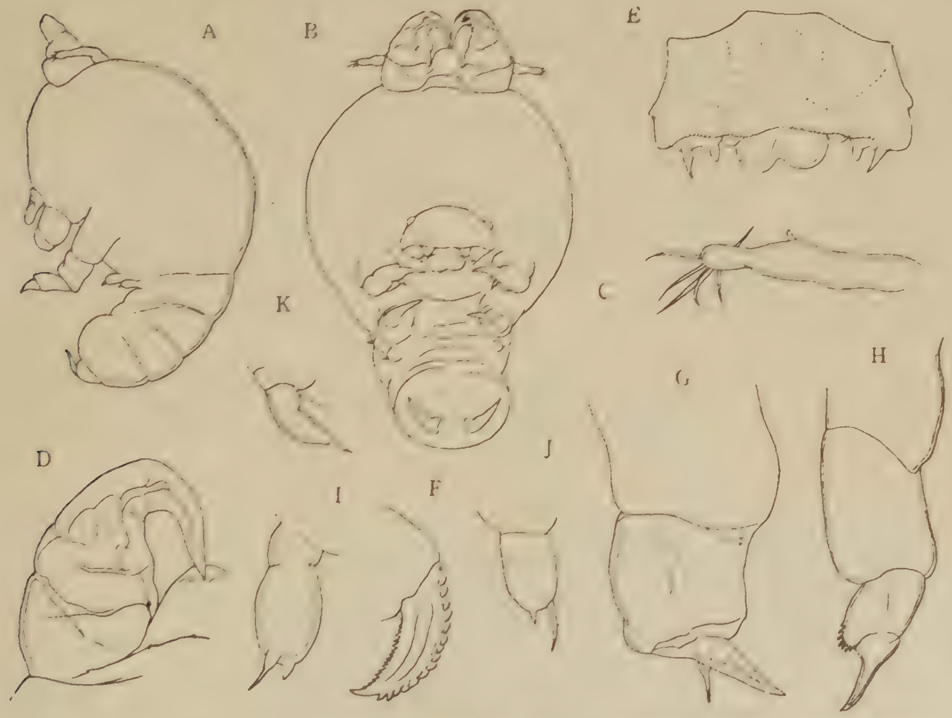

Fig. 10. *Acanthochondria incisa* n. sp., male. A, lateral view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, oral region, showing labrum, mandibles and maxillae; F, mandible; G, 1st maxilliped; H, 2nd maxilliped; I, 1st leg; J, 2nd leg; K, caudal ramus. A ×102, B ×120, C–E, G, H, K ×340, F, I, J ×486.

ween consecutive 2 of succeeding segments comparatively well defined. Abdomen small, hemispherical. First antennae moderately long, unjointed, but with a constriction a little behind apex, and bearing a short cirrus on anterior border about the middle besides with stiff apical setae. Second antennae robust, with broad base and sharply pointed apex which is incurved and reaches semicircular frontal plate. Labrum prominent, with tuberculated free margin. Mandibles falcate, with marginal teeth stouter on posterior side than on anterior. Maxillae semicircular, with a sharp longer outer and a blunt shorter inner spines. Exopod of 1st maxillipeds styliform, tapering distally, with straight axis and entire borders; endopod ending in spiniform apex. Claw of 2nd maxillipeds slender than exopod of preceding limbs, only slightly curved, without accessory spine; penultimate joint with oval distal bulge marginated with a few spinules. First pair of leg rudiments indistinctly jointed by incomplete line of border present near base, distal joint oblong ovate, tipped with 2, longer and shorter spines. Second pair shorter than 1st, unjointed, similarly with 2 unequal spines. Caudal rami elongate conical, terminating in a feeble spine.

Remarks: In the general appearance of female body, especially in the shape and size of the composite (3rd and 4th) segment, the present species resembles *phycidis* (Rathbun)[16,22], *depressa* (T. Scott)[14,18,19,22] and *brevicorpa* Yü[24]. It is different from *phycidis* in that the named segment is relatively broader but more deeply incised and has the posterior processes turned inwards, in that the head is not produced in front, and in that the genital segment and abdomen are much smaller; from *depressa* in the first 2 free segments having unequal width, in the legs lacking surface prickles, and in the feebler antennules, basal joint of which is devoid of internal bulge; from *brevicorpa* in the head having entire sides, and in that the outer branches of 2 pairs of legs have apices rounded without developing any accessory tubercles.

Acanthochondria longifrons n. sp.

(Figs. 11 & 12)

Occurrence: On the gills of *Antennarius tridens* (T. & S.) taken at Tyôsi Market, Tiba Prefecture, June 9, 1943, by myself. Female inserts head into soft tissue of gill arch, trailing body alongside the gill filaments. 2 female specimens each accompanying a dwarf male, one of them being selected as holotype.

Female: Holotype. Length from antennae to egg tubes 5.96, from head to abdomen 3.9, head 1.29×1.19, 1st thoracic segment 0.43×0.95, 2nd segment 0.71×1.19, 3rd segment 0.81×2.00, 4th segment, length on midline 0.57, same including posterior process 1.00, width 1.76, genital segment 0.19×0.48, abdomen 0.19×0.19, egg tubes 2.14 long. Color pale yellowish brown in alcohol.

Cephalothorax rather large in size, occupying approximately 1/3 of body length, longer than wide, pyriform, being narrowed anteriorly and plump poster-

Fig. 11. *Acanthochondria longifrons* n. sp., female. A, dorsal view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, labrum, inner side; F, mandible; G, maxilla; H, 1st maxilliped; I, apical part of same; J, 2nd maxilliped; K, apical part of same; L, 1st leg; M, 2nd leg; N, genital segment and abdomen, ventral view; O, caudal ramus, lateral view. A, B ×14, C, H, J ×170, D, N ×83, E–G, I, K, O ×340, L, M ×41.

iorly. First thoracic segment distinctly bordered from and narrower than cephalothorax. Second segment a little wider and longer than 1st, with sides continuous to bases of legs without demarcation, and more or less indistinctly bordered from adjoining segments both in front and in the rear. Third and 4th segments confluent, with boundary indicated merely by deep lateral notches at about the middle. Confluent segment relatively short, less than half as long as body, wider than long, with sides well rounded both before and behind the notches; posterior part narrowing backwards and forming a pair of short blunt lobes, which extend a trifle beyond abdomen. Genital segment hemispherical, carrying at end a globular abdomen.

First antennae sausage-shaped, non-articulated, with a few feeble terminal and subterminal hairs. Second antennae well-curved, sharp at apex. Labrum with rows of minute tubercles along free margin. Mandibles with teeth stouter and coarser on posterior edge than on anterior edge where these are obsolete for a short distance behind apex. Maxillae elongate, fusiform, tipped with 2 unequal spines, longer member of which has a node at the middle. Exopodite of 1st maxillipeds attenuating, almost straight, recurving only near tip and with well-developed teeth on inner margin; endopod short, with sharp spiniform apex. Claw of 2nd maxillipeds falcate, bluntly pointed, with an inner tubercle representing remnant of accessory branch; 2nd joint has inner distal expansion lunular, fringed with double row of spinules which passes to inner border of joint through a short interruption. Two pairs of legs unequal in size, posterior being larger; outer branch blunt, while inner branch more or less acute in both pairs. (Left 2nd leg with extremity undivided, remaining in a condition of simple tubercle, probably due to an injury in the lifetime.) Genital segment furnished with unguiform rudiments of legs, 1 on each side of mid-ventral line. Caudal rami conical, bluntly pointed, inconspicuously 2-jointed, and armed with 2 basal spines, arranged 1 on anterior and posterior sides respectively. Egg-tubes relatively short, sausage-shaped.

Male: Dwarf, colorless, strongly crooked. Length 0.38, head, length on dorsal side 0.27, thickness 0.28, free segments, length on dorsal side 0.28, same on ventral side 0.12, thickness in 1st segment 0.15, same in 4th segment 0.12.

Head globular, with ventral surface somewhat uneven in preoral region. Free segments with 5 more or less indistinct segmentation lines; 1st segment bordered from head by similar line. Frontal plate prominent, of U-shape. First antennae 2-jointed; distal joint short, with apical bunch of a few setae, proximal joint much longer, with 2 triangular cirri on anterior border and 2 setae at end. Second antennae as usual, with reflexed part slender and sharp. Labrum notched at centre, with a series of short parallel ridges which are perpendicular to free border. Mandibles not much curved, serrated on each border. Maxillae ovate,

with 2 short subequal spines. Exopod of 1st maxillipeds nearly straight, ensiform, narrowing and pointing to extremity, and destitute of marginal serration; endopod rod-shaped, with feeble apical spine. Claw of 2nd maxillipeds with sharp slightly curved end, without accessory process; penultimate joint has terminal bulge thickly dentate, row of denticles extending to middle of inner border of joint, then disappearing. First leg rudiments expanded distally, forming triangular lobe at inner angle and rounded lobe with a spine at outer angle. Second legs narrower than 1st, with short additional spine instead of triangular lobe. Caudal rami acuminating, somewhat crinkled, 2-jointed, non-hairy; apical joint far longer than basal.

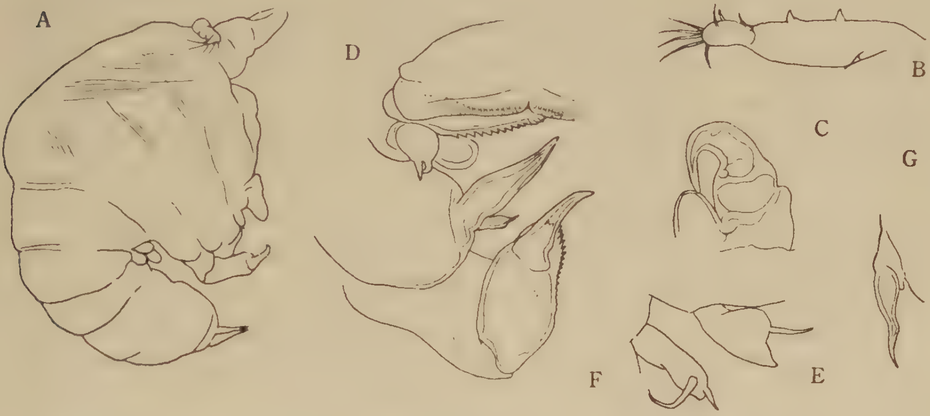

Fig. 12. *Acanthochondria longifrons* n. sp., male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, labrum, mandible, maxilla and 1st and 2nd maxillipeds in situ; E, 1st leg; F, 2nd leg; G, caudal ramus. A ×120, B, D–G ×340, C ×170.

Remarks: The present species is nearly allied to *incisa* n. sp. described in the preceding section, *hazekuti* YAMAGUTI[23], *showi* Yü[24], *brevicorpa* Yü[24], *briani* (Yü and Wu)[25], and *phycidis* (RATHBUN) in the female having a relatively large-sized head and a broad confluent segment. But it is distinguished from *hazekuti* by the pronouncedly elongate head and by the distinctness of demarcation between the 3rd and 4th segments; from *brevicorpa* by the absence of accessory tubercle on the outer rami of the legs, by the shortness of thoracic processes, as well as by the head lacking lateral constrictions which are present in *brevicorpa*; from *briani* and *phycidis* by that the confluent segment is shorter and broader and possesses deeper lateral incisions, and again from the latter of these by the genital segment and the abdomen being smaller. As regards the differences between *longifrons* and *incisa* there will be no need of superfluous words.

Acanthochondria fissicauda n. sp.
(Figs. 13 & 14)

Occurrence: Hanging on wall of buccal cavity of *Callionymus richardsoni* BLEEKER taken at Tyôsi Fish-Market, June 9, 1943, by myself. 6 females each associated with a dwarf male. Out of them a female is selected as type.

Female: Holotype. Length from head to egg tubes 5.26, same to abdomen 3.48, same to thoracic processes 4.04, head 0.96×0.96, 1st thoracic segment 0.20×0.67, 2nd segment 0.46×0.91, 3rd plus 4th segment, length on mid-dorsal line 1.65, same on either side 1.96, width 1.70, genital segment 0.13×0.30, abdomen 0.09×0.17, egg tube, length 2.00. Whitish, egg strings same in color in preservative.

Head occupying almost a quarter of entire length, pyriform, having narrowed anterior and plump posterior regions, and convex on dorsal side. First thoracic segment short, narrower than head, transversely linear, distinctly bordered both in front and back. Second segment more than twice as long as 1st and almost as wide as head; anterior end narrowed to form a short neck, sides well rounded, and posterior border distinct. Third and 4th segments coalesced leaving no trace of coalescence. Coalesced segment about as long as rest of body on midline, a little longer than wide, with nearly parallel but slightly undulated sides, posterior corners produced into blunt deltoid lobes which interpose between each other a V-shaped sinus. Genital segment cup-shaped, small in size, bearing further smaller abdomen which is globular and ventrally turned.

First antennae filiform, attenuating terminally, 2-jointed; 1st joint occupying almost entire length, with 1 or 2 feeble hairs at end, and surmounted with much reduced mammiform sparingly hairy 2nd joint. Second antennae hook-like as usual, with sharp apex. Labrum with tiny tubercles on border, Mandibles falcate, with 2 edges thickly fringed by tall acuminate teeth which are undeveloped for some distance behind sharp tip on anterior edge and for a shorter distance on posterior edge. Maxillae roughly triangular, armed with a robust apical spine associated close to its base with a short feeble one. Exopod of 1st maxillipeds relatively short and broad, slightly recurved, with marginal teeth conspicuous only in terminal portion but reduced in basal portion; endopod rod-shaped, terminating in spiniform apex; end joint of sympod short, rather broad. Claw of 2nd maxillipeds as stout as exopod of preceding limbs, narrowed towards apex, and with a rudiment of spine on inner border; penultimate joint expanded at inner distal angle into a semicircular lobe, circumscribed with uniserial well-developed spines. Two pairs of legs equal in structure, but unequal in size, posterior pair being the larger. In both pairs outer branch blunt, while inner branch somewhat acute, the two making a right angle. No limb rudiment on genital segment. Caudal rami composed of bulbous base with a feeble inner hair

Fig. 13. *Acanthochondria fissicauda* n. sp., female. A, dorsal view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, labrum; F, mandible; G, maxilla; H, 1st maxilliped; I, apical part of same; J, 2nd maxilliped; K, apical 2 joints of same; L, 1st leg; M, 2nd leg; N, genital segment and abdomen, dorsal view; O, same, ventral view; P, same, lateral view; Q, caudal ramus. A, B ×15.3, C, G, H, J, Q ×170, D ×120, E, F, I, K ×348, L–P ×41.

and much narrowed conical apex ending in a spine. Egg tubes banana-shaped, short and broad.

Male: Dwarf, colorless, translucent. Length 0.48, head, length 0.29, thickness 0.28, free segments, length on dorsal line 0.34, 1st segment of same, thickness 0.19, last segment, same 0.11.

Head globular, more convex dorsally than ventrally, with posterior border well defined. Rest of body divisible into 5 segments by distinct lines of borders and constrictions. First segment broader than succeeding ones; genital segment and abdomen inseparable from each other. Frontal plate spatulate and truncate on free border. First antennae unjointed, filiform, attenuating towards apex where they bear a number of setae. Second antennae with broad base and reflexed tip rapidly acuminating to a sharp point. Mandibles strongly curved, with denticles feebler on anterior border than on posterior border. Maxillae elongate narrow lamellae, with 2 unequal spinules at rounded end. First maxillipeds with sympod short and broad; exopod beak-like, rapidly tapering to blunt point, destitute of denticles, endopod styliform, with sharp apex. Claw of 2nd maxillipeds slender, feeble, curved only near apex, without accessory process; penultimate joint with sparse row of spinules on inner border as well as on distal border, 2 rows intersecting at a right angle. First leg rudiments broad, obliquely truncate at end, where they carry a rather long and a rudimentary spines. Second legs narrower than preceding, oblong ovate, with a single long spine. Caudal rami consist of short basal and elongate styliform apical joints; basal joint has 2 hairs, one on ventral and the other on dorsal sides.

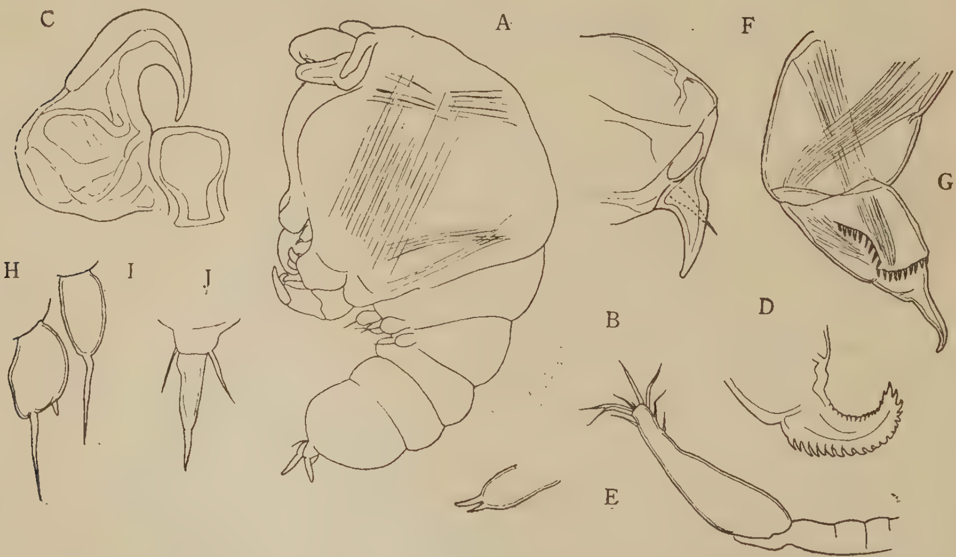

Fig. 14. *Acanthochondria fissicauda* n. sp., male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, mandible; E, maxilla; F, 1st maxilliped; G, 2nd maxilliped; H, 1st leg; I, 2nd leg; J, caudal ramus. A ×120, B, C, E–J ×348, D ×486.

Remarks: In that the lateral notches between the 3rd and 4th segments which are fused together are more or less obsolete, the new species is closely allied to *hazekuti* YAMAGUTI[23], *shawi* YÜ[24] and *wui* YÜ[24]. It can be distinguished from these by the complete absence of the named notches, by the broader divergent posterior lobes of the 4th segment, and by the first 2 thoracic segments showing distinct segmentation lines. In general appearance, in the distinctness and proportional sizes of the first 2 thoracic segments, the new species bears a certain resemblance to *briani* (YÜ & WU)[25] also. But the complete union of the 3rd and 4th segments in the former is, above all things, the most remarkable feature in differentiating the two.

Acanthochondria glandiceps n. sp.

(Figs. 15 & 16)

Occurrence: Attached to gill filament of *Hippoglossoides dubius* (SCHMIDT) collected at Wakkanai, Hokkaidô, by Dr. K. KURONUMA, May 31, 1943. Numerous specimens; out of them a female is selected as holotype.

Female: Holotype. Color pale whitish yellow, egg cases yellowish brown in preservative. Length from head to egg tubes 13.0, same to abdomen 8.40, head 2.35×1.49, 1st thoracic segment 1.38×1.15, 2nd segment 0.82×1.92, 3rd plus 4th segment 3.80×2.29, same, length inclusive of posterior processes 4.60, genital segment 1.15×1.96, abdomen 0.82×1.15, egg tubes, length 5.52.

Head oblong ovoidal, longer than wide, with antero-dorsal surface more strongly chitinized than rest; posterior region insensibly swollen and demarked from succeeding segment by surface groove. Thoracic segmentation indicated by shallow grooves also. First segment relatively long, cylindrical, and slightly narrower than head. Second segment shorter but wider than foregoing, attaining 1 and a quarter of head width, with blunt cuneiform sides, continuing to succeeding segment through a constriction. Third and 4th segments entirely fused together to form an oblong segment which is less than half as long as body, about 1 and half times as long as wide, and rather thick dorso-ventrally. Fused segment has gracefully curved anterior shoulders and slightly undulated sides, central depression of which indicates site of fusion; posterior end produced into a pair of short triangular lobes, inserting V-shaped median sinus. Genital segment very small, wider than long, hemispherical. Abdomen further smaller, globular.

First antennae slightly curved, broad in relation to length, unjointed, tipped with 2 or 3 short feeble hairs. Second antennae with broad base, terminal part acuminating to a sharp point, but not so strongly curved as in ordinary case. Blade of mandibles well curved, thickly dentate on 2 edges extending over their entire lengths; denticles very sharp and uniform in size. Maxillae semicircular,

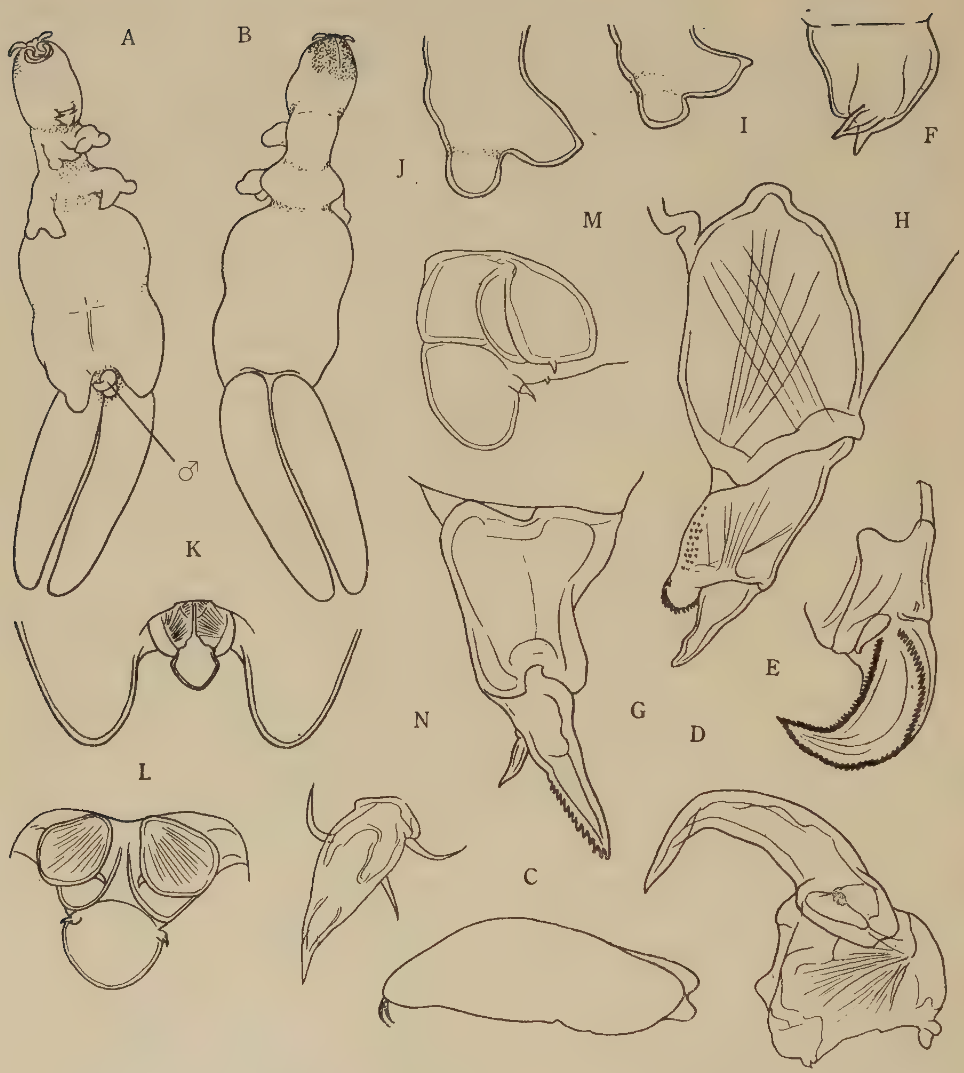

Fig. 15. *Acanthochondria glandiceps* n. sp., female. A, dorsal view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, mandible; F, maxilla; G, 1st maxilliped; H, 2nd maxilliped; I, 1st leg; J, 2nd leg; K, genital segment and abdomen, dorsal view; L, same, ventral view, further enlarged; M, same, lateral view; N, caudal ramus. A, B ×5.8, C, I–K ×18.7, D ×83, E–H ×170, L, M ×41, N ×120.

with 2 conical acicula. Exopod of 1st maxillipeds robust, nearly straight, narrowing to acute apex, and with inner border dentate on its distal half; endopod styliform, pointed. Claw of 2nd maxillipeds feebler than exopod of 1st, tapering towards blunt end, and with a low inconspicuous bulge on inner border which represents remnant of accessory spine; 2nd joint with a rounded bulge fringed with spinules at inner distal angle and longitudinal rows of tubercles on surface along inner margin. Two pairs of legs similar in structure, but dissimilar in

size, posterior pair being larger. In both pairs outer branch rounded at apex, while inner branch angular at inner corner, which is somewhat acuter in 1st pair than in 2nd. Minute unguiform rudiments of legs present on ventral surface of genital segment close to midline and internally to paired oval muscle swellings. Caudal rami conical, sharply pointed, indistinctly 2-jointed; base with 2 ventral and 1 dorsal hairs and apex with terminal spine indistinguishable from ramus itself.

Male: Dwarf, colorless, pellucid, not very much crooked. Length 0.66, head 0.37×0.36, its thickness 0.35, free segments 0.26×0.17, thickness in 2nd segment 0.18.

Head ovate, more convex dorsally than ventrally. Frist thoracic segment indistinctly defined from head and rather thick dorso-ventrally. Segmentation of rest of body comparatively conspicuous on all sides, with 5 segments distinguishable. Frontal plate prominent, with rounded apex. First antennae indistinctly 3-jointed,

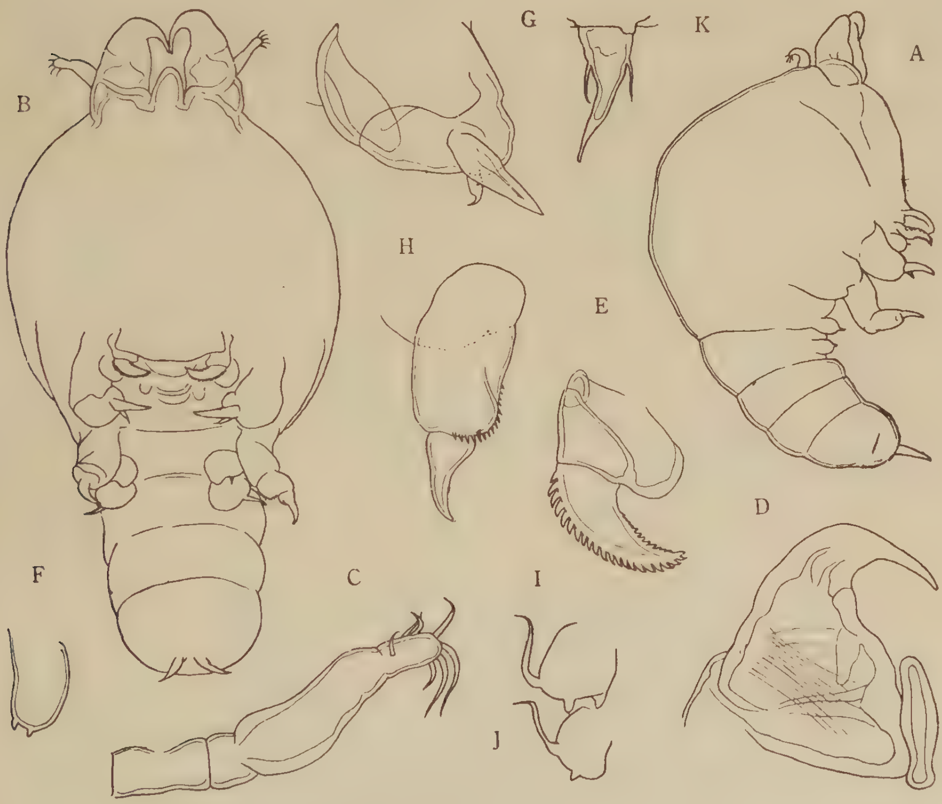

Fig. 16. *Acanthochondria glandiceps* n. p., male. A, lateral view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, mandible; F, maxilla; G, 1st maxilliped; H, terminal 2 joints of 2nd maxilliped; I, 1st leg; J, 2nd leg; K, caudal ramus. A ×83, B ×120, C, D, G-K ×340, E, F ×486.

1st and 3rd joints rather short, 2nd occupying far greater part of length; 3rd with a number of terminal and subterminal setae, others non-setaceous. Second antennae with broad base, but abruptly narrowed from curving point towards apical portion which is relatively short. Labrum without tuberculation. Mandibles falcate, with teeth on posterior edge stout, while those on anterior edge rather feeble and almost reduced near base. Maxillae elongate lobes with rounded apex, surmounted with 2 rudimentary spines. Exopod of 1st maxillipeds acute, ensiform, without bordering teeth, endopod rod-shaped, ending in feeble spine; sympod broad. Claw of 2nd maxillipeds almost as large as exopod of preceding, attenuating to curved and sharp extremity; penultimate joint oblong, with a low lunular inner distal expansion fringed with spinules ranging from middle of distal border to same of inner border. Leg rudiments broad, distorted oval in shape. First pair slightly larger than 2nd, with 1 long and 2 rudimentary tuberculiform hairs; 2nd with 1 long and 1 rudimentary hairs. Caudal rami elongate conical, acuminating, with apical spine undifferentiated from joint and 2 hairs a little upward from base, disposed 1 on ventral and dorsal sides respectively.

Remarks: In the general appearance of body, or in the proportion of segments, the present species bears a certain resemblance to *galerita* (Rathbun)[16,22], *sixteni* (Wilson)[9,21], *epachthes* (Wilson)[14,20], *cornuta* (Müller)[1-3,6,9,10,13-15,17,19,22], and *glandigenitalia* (Yü & Wu)[25], but can be distinguished from these, for the first ground, by the character of the segment formed by union of the 3rd and 4th. The segment of the new species is different from that of the 1st of the named species in being relatively wider, from that of the 2nd and 3rd in lacking border line between the constituent segments, from that of the 4th in having lateral depressions and much wider posterior processes, and from that of the 5th in the possession of shallower depressions and shorter but broader processes. Moreover, in the new species the 2nd thoracic segment is longer than in *sixteni* already known from Japan and in *epachthes* and the 1st antennae are filiform unlike those of *galerita* which are lamellar.

Acanthochondria constricta n. sp.

(Figs. 17 & 18)

Occurrence: On *Doederleinia berycoides* (Hilgendorf), taken at Owase, Mie Prefecture, December 9, 1952; anterior part of female body is buried in tissue of floor of buccal cavity of host; 3 females each with a male. A female out of them is selected as holotype.

Female: Holotype. Body and egg strings whitish in color. Length from head to egg tubes 10.05, same to abdomen 6.05, same to posterior processes 6.22, head 0.95×1.26, thickness 1.21, 1st thoracic segment 0.32×0.95, 2nd segment

Fig. 17. *Acanthochondria constricta* n. sp., female. A, dorsal view; B, ventral view; C, head and free thorax segments, dorsal view; D, 1st antenna; E, 2nd antenna; F, mandible; G, same, further enlarged, posterior view; H, maxilla; I, 1st maxilliped; J, apical part of same, further enlarged; K, 2nd maxilliped; L, apical part of same, further enlarged; M, 1st leg; N, 2nd leg; O, genital segment and abdomen, dorsal view; P, same, ventral view; Q, same, lateral view; R, caudal ramus, lateral view. A–C ×12.7, D, H, I, K ×120, E ×83, F, R ×170, G, J, L ×340, M, N ×18.7, O–Q ×41.

0.95×1.58, 3rd plus 4th segment 3.26×2.48, same including posterior processes 4.05 long, egg tubes 4.00 long.

Body elongate. Head ovoidal, somewhat more convex dorsally than ventrally, and with slight bulges close to bases of maxillipeds. Segmentation of thorax largely obsolete. First segment short, narrower than and distinct from head, and in form of a ring connecting this with 2nd segment. Second segment wider than head, with well-rounded sides, and constricted both in front and in the rear. Third and 4th segments nearly twice as long as rest of body, a trifle over 2/3 as wide as long and demarked from each other by moderately deep wedge-shaped lateral notches, before and behind which sides are widely rounded though making insignificant angle just in front. Posterior end of 4th segment produced into short narrow blunt lobes, which turn slightly inwards and interpose a trapezoid sinus. Genital segment small, wider than long, hemispherical. Abdomen further smaller, conical, with blunt apex, and strongly bending ventrally.

First antennae filiform, curved, non-articulated, with 1 or 2 minute tubercles on anterior border besides ordinary apical hairs. Second antennae with swollen base and columnar shaft, terminal part of which curves at a right angle and abruptly narrows toward pointed end. Blade of mandibles curved in a sickle-shape, with its tip sharp, recurved in an opposite direction; denticles on anterior edge smaller in size than those on posterior. Maxillae nearly triangular, carrying short spine at end and further shorter one a little behind. Exopod of 1st maxillipeds not obviously curved, relatively broad, and with sharp extremity; marginal teeth beginning a little behind apex and rapidly diminishing in size to disappear at about the middle. Endopod relatively well-developed, with much narrowed spiniform apex. Claw of 2nd maxillipeds as long as exopod of preceding limbs but slenderer, bluntly pointed, and with a rudimentary spine on inner border. Penultimate joint with semicircular terminal expansion, fringed with double row of sharp spinules; inner border with another row of spinules. First legs smaller than 2nd in size, with branches diverging not very much, outer member of these blunt, while inner member with acute inner distal angle. Second legs with outer branch blunt and inner branch somewhat acuter, yet blunt, both making a right angle to each other. Caudal rami conical, acuminating, with terminal spine undifferentiated; basal hairs 3 in number, disposed 2 on ventral and 1 on dorsal sides. Egg tubes elongate, 2/3 as long as body.

Male: Pygmy, not strongly crooked, colorless and translucent. Length 0.53, head, length on dorsal line 0.32, same on ventral line 0.28, thickness 0.30, free segments, length on dorsal line 0.33, same on ventral line 0.21, 1st segment, thickness 0.18, last segment, same 0.10.

Head oblong ovate, not clearly defined posteriorly from 1st thoracic segment. Succeeding segments gradually narrower backwards, showing 4 more or less

distinct border lines. Frontal plate widely round in front, narrowed in behind, making an inverted triangle. First antennae filiform, somewhat narrowed near apex at which they carry a few hairs. Second antennae swollen at base, with hook relatively thick but ending narrowed tip. Mandibles lunular in shape; denticles small in number, stouter and more coarsely arranged on posterior edge than on anterior. Maxillae obovate, contracted at base, with 2 subequal spines. Exopod of 1st maxillipeds slightly curved, narrowing to blunt point, and with entire border; endopod terminating in spiniform apex. End joint of 2nd maxillipeds slenderer than exopod of previous limbs, nearly straight, styliform, with sharp apex, and without accessory process; spinules on penultimate joint uniseriate and extending from outer distal angle of joint to middle of inner border. Two pairs of legs resembling each other, ovate, with contracted base, and tipped with 2 unequal spinules. Caudal rami indistinctly 2-jointed, consisting of shorter columnar basal and longer styliform terminal joints, and with 2 ventral and 1 dorsal setae at base.

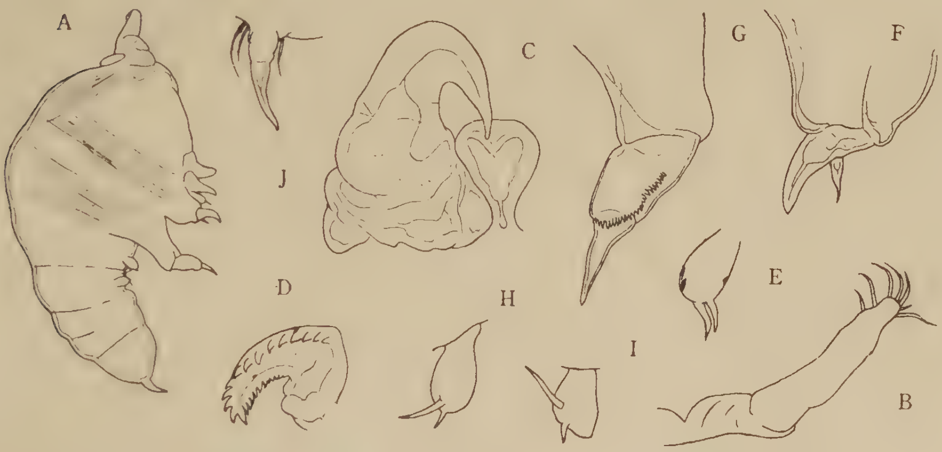

Fig. 18. *Acanthochondria constricta* n. sp., male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, mandible; E, maxilla; F, 1st maxilliped; G, 2nd maxilliped; H, 1st leg; I, 2nd leg; J, caudal ramus. A ×83, B, C, E–J ×340, D ×486.

Remarks: The present species is more or less nearly allied to *epachthes* (Wilson)[14, 20], *exilipes* Wilson[22], *cornuta* (Müller)[1–3, 6, 9, 10, 13–15, 17, 19, 22], *grandigenitalia* (Yü & Wu)[25], and *depressa* var. *oblonga* (T. & A. Scott)[19], as well as to *glandiceps* described above, in general constitution of the body. It is distinguished from *epachthes* and *exilipes* by the entire absence of segmental line between the 3rd and 4th segments, and by the shortness of posterior processes; from *cornuta* by these segments being demarked by lateral notches; from *grandigenitalia* by the notches being much deeper and by the relatively longer 1st and 2nd segments; and finally from *oblonga* by the unequal width of these last named

segments and by the filiform 1st antennae. As to the differences between the present species and *glandiceps*, there will be no necessity of special mention.

Acanthochondria spirigera n. sp.
(Figs. 19 & 20)

Occurrence: On *Lophiomus setigerous* (VAHL) collected at Tyôsi, Tiba Prefecture, June 9, 1943, at Amatu, Tiba Prefecture, April 24, 1946, and at Owase, Mie Prefecture, December 9, 1952, November 18, 1953, and on *Cherolophius moseleyi* REGAN, at Owase, September 22, 1951. Numerous specimens clinging to roof and floor of buccal cavity of host. A female on the former host taken at the first named locality is selected as holotype.

Female: Holotype. Rather large in size, whitish in color, with yellow egg strings. Length from head to abdomen 10.7, same to posterior processes 11.1, same, including egg strings 13.5, head, length, width, and thickness 1.8×1.8×1.56, 1st thoracic segment 0.9×1.5×1.56, 2nd segment 0.9×2.3×1.71, 3rd plus 4th segment 6.8×4.7×2.6, same, length including posterior processes 7.93, genital segment 0.30×0.58, abdomen 0.40×0.33, egg string, extended, length 10.05.

Head relatively small in size, ovate, convex both dorsally and ventrally, and bulged close to bases of 2nd maxillipeds. First thoracic segment distinct from and narrower than head, divided into 2 subequal regions by transverse rib which is interrupted on mid-dorsal face; anterior region bearing legs on ventral side. Second segment distinctly bordered both in front and back, transversely linear in dorsal aspect, with well-rounded lateral sides. Third and 4th segments united into an enormous segment which occupies greater part of body and is swollen dorso-ventrally. United segment contracted at frontal end and gradually narrowed caudally forming a pair of linguiform elongations; 2 sides with cuneiform notches at about the middle, indicating boundary between component segments and widely curved both forwards and backwards. Genital segment very small in size, hemisperical; abdomen further smaller, spherical, and bent ventrally.

First antennae filiform, curved, obscurely 4-jointed, and with a few feeble apical setae. Second antennae powerful hooks having swollen base, rather long straight shaft, and sharp curved apex. Labrum without tuberculation, but associated close to each side with an oval protuberance. Mandibles falcate, rather thick at base, and with edges of blade regularly and uniformly cut into sharp denticles. Maxillae triangular though more or less irregular in outline, with stouter outer and feebler inner spines. They bear, further, a small linguiform basal lamina on surface close to inner corner and an oval sparingly ciliated one just inside base (as regards the homology of these laminae I can make nothing of what they are). Exopod of 1st maxillipeds tapering, only slightly recurved, with marginal teeth uniform in size, and undeveloped for a considerable distance from base; endopod

Fig. 19. *Acanthochondria spirigera* n. sp., female. A, dorsal view; B, ventral view; C, lateral view; D, 1st antenna; E, 2nd antenna; F, oral region, showing labrum, mandibles and maxillae in situ; G, mandible; H, maxilla; I, 1st maxilliped; J, apical part of same; K, 2nd maxilliped; L, apical part of same; M, 1st leg; N, 2nd leg; O, abdomen, dorsal view; P, same, ventro-lateral view; Q, appendage on genital segment; R, caudal ramus. A, B ×6.7, C ×5.1, D–F ×83, G–J, L ×170, K, Q, R ×120, M, N ×18.7, D ×27, P ×41.

proportionally narrow, ending in a spinule. Claw of 2nd maxillipeds shorter and feebler than exopod of 1st maxillipeds, slightly curved, with a low tubercle behind blunt tip, representing rudiment of accessory process, and with a short row of minute hairs further back and across axis of claw. Penultimate joint has a semicircular distal expansion marginated with row of spinules which extends along inner border as far near as base of joint. Both pairs of legs similar in structure. Outer ramus claviform with swollen end, while inner ramus acute at inner angle; stalk of 1st pair bulged close to base of rami, that of 2nd longer, not much bulged. Minute unguiform rudiments of legs present on genital segment close to mid-ventral line. Caudal rami 2-jointed, composed of pointed conical apical joint and oval basal joint with short inner hair. Egg strings elongate, spirally twisted; eggs pluristichous.

Male: Dwarf, curved ventrally, colorless and pellucid. Length 0.58, head, length on dorsal side 0.37, same, on ventral side 0.32, width 0.39, thickness 0.30,

Fig. 20. *Acanthochondria spirigera* n. sp., male. A, lateral view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, labrum; F, mandible; G, maxilla; H, 1st maxilliped; I, 2nd maxilliped; J, 1st leg; K, 2nd leg; L, caudal ramus. A, B ×120, C–E, G–L ×340, F ×485.

free segments, length on dorsal side 0.29, same, on ventral side 0.25, 2nd segment, width and thickness 0.19×0.14, 4th segment, same 0.14×0.12.

Head ovoidal, strongly convex on dorsal side, uneven on ventral side. First thoracic segment undifferentiated from head. Rest of body more or less distinct in articulation, with 4 segments discernible. Frontal plate prominent, triangular, with blunt apex. First antennae obscurely 2-jointed, basal joint elongate, occupying greater part of antennal length, while apical joints very short, tipped by relatively long setae. Second antennae with swollen base and broad axis which is much narrowed beyond curving point acuminating to a sharp apex. Labrum with a row of short spinules along free margin. Mandibles falciform, pointed, with marginal denticles sharp and more numerous than in ordinary case. Maxillae elongate oval, with 2 subequal flexible spines. Exopod of 1st maxillipeds slightly curved, tapering to a pointed end, with borders entire, endopod short, styliform, and sympod strongly bulged terminally. Claw of 2nd maxillipeds subequal to exopod of preceding appendages in shape and size and without any trace of accessory spine; 2nd joint with a low, crescent distal expansion, fringed with a single row of spinules, which reaches a vicinity of base of joint. First legs oblong, with 1 long hair and 2 short spines on distal margin. Second legs narrower than 1st and with 1 long and 1 rudimentary hairs. Caudal rami slender, spiniform, consisting of short basal and elongate pointed apical joints; basal joint with a single short hair.

Remarks: Although the new species is more or less nearly allied to *oralis* YAMAGUTI[23], *cornuta* (MÜLLER)[1–4, 6, 9, 10, 13–15, 17, 19, 22], *depressa* var. *cblonga* (T. & A. SCOTT)[19], and *epachthes* (WILSON)[14, 20] in the general body form, it is recognized, above all characters, by the possession of spirally twisted egg-strings.

Acanthochondria bicornis n. sp.

(Figs. 21 & 22)

Occurrence: 2 females each with a dwarf male on *Helioclenus dactylopterus* (DE LA ROCHE), off Kumano District, December 18, 1952; a female without male on *Zenion japonicum* KAMOHARA, at a depth of 100–200 fathoms off Minabe, Wakayama Prefecture, May 1943, 2 females each with a male on *Setarches longimanus* (ALCOCK & MCGILCHRIST), at Seto, Wakayama Prefecture, date unknown. All specimens attached to upper side of gill arches, with anterior part of body (from 2nd segment forward) buried in tumor formed by host tissue. They clasp gill arch bone with their 2nd antennae, sharp tips of which are thrust deep into bone. A female from *Helioclenus* is selected as type.

Female: Holotype. Color whitish, somewhat pellucid. Length up to posterior processes of thorax 5.1, same to abdomen 5.0, head 1.38×0.81, 1st

thoracic segment 0.48×0.67, 2nd segment 0.48×1.24, 3rd plus 4th segment, length on midline 2.33, same, on lateral region 2.66, width 1.95, genital segment 0.14×0.33, abdomen 0.19×0.19.

Head less than twice longer than wide, oblong, having parallel sides, convex dorsally, but depressed ventrally; ventral surface produced at antero-lateral corners into short finger-shaped lobes. First thoracic segment narrower than head,

Fig. 21. *Acanthochondria bicornis* n. sp., female. A, dorsal view; B, ventral view; C, 1st and 2nd antennae in situ; D, labrum; E, mandible; F, maxilla; G, 1st maxilliped; H, 2nd maxilliped; I, 1st leg; J, 2nd leg; K, abdomen with caudal rami. A, B ×14, C, K ×120, D, F ×83, E, G, H ×340, I, J ×41.

a little shorter than wide, with distinct boundaries both in front and back. Second segment as long as 1st, wider than head, with round sides, and bordered from following segment by deep triangular lateral sinuses, but with segmentation line obliterated. Third and 4th segments united together leaving no trace of union. United segment a broad fusiform, having widely arcuate sides, swollen dorso-ventrally, and with a pair of short narrow blunt posterior lobes, which interpose between each other a quadrate sinus. Genital segment small, hemispherical. Abdomen further smaller, globose.

First antennae unjointed, short, clavate, provided about the middle with 2 rounded tubercles arranged proximo-distally and surmounted each by a tiny spine, in addition to bearing a few feeble hairs near apex. Second antennae bulged at base not very much, with terminal part beyond reflecting point sharp and long, making an acute angle with columnar shaft. Oral appendages crowded together at posterior end of head. Labrum marginated with a row of sharp spinules. Mandibles broad at base, with blade falcate, 2 edges dentate along their entire lengths. Maxillae triangular, with thick but sharp spine at apex and much shorter unguiform one just behind. Exopod of 1st maxillipeds recurved in falciform and closely dentate on posterior border; endopod rod-shaped, ending in a feeble spine. Terminal joint of 2nd maxillipeds with straight axis, bulged at base, acuminating to a sharp tip, and with accessory process obsolete; penultimate joint with oval terminal expansion, circumscribed with fine spinules. Two pairs of legs unequal in size and in structure. First pair shorter than 2nd, with outer branch short, knob-like, somewhat turned inwards, and inner branch longer, columnar, directed backwards. Outer branch of 2nd pair resembling that of 1st and internally turned, while inner branch far longer, cylindrical and projecting inwards. Apices of branches well-rounded in all legs. Caudal rami 2-jointed; proximal joint swollen, oval, furnished with 1 dorsal and 1 ventral hairs, distal joint slender, spiniform. Egg tubes missing.

Male: Body crooked ventrally, colorless and translucent. Length 0.46, head, length on dorsal side 0.26, same, thickness 0.24, free segments, length on dorsal side 0.19, same, on ventral side 0.14, thickness in 2nd segment 0.10, same in last segment 0.08.

Head globular, strongly convex on dorsal side, but less so on ventral side. First thoracic segment inseparable from head. Following segments more or less distinct from one another by faint surface grooves. Frontal plate triangular. First antennae filiform, unjointed, tipped with a few hairs and bearing 2 minute tubercles on posterior border. Second antennae with reflexed part relatively broad, but sharply pointed, base bulged, more or less crinkled. Labrum with a row of minute papillae along margin. Mandibles falciform, with denticles rather sharp, somewhat feebler on inner edge than on outer. Maxillae elongate oval, tipped

with 2 sharp spines of unequal lengths. Exopod of 1st maxillipeds beak-like in shape, slightly curving, with pointed apex and entire borders; endopod rod-shaped, ending in a spinule. Terminal joint of 2nd maxillipeds broad at base, rapidly tapering to curved pointed tip, and without accessory branch; penultimate joint with lunular distal bulge encircled by a row of spinules. Two pairs of legs similar in size and in structure, elongate oval, bearing a long stiff hair and a very short rudimentary spine at end. Caudal rami unjointed, styliform, ending in sharp point, and with a short hair near base on dorsal and ventral sides respectively.

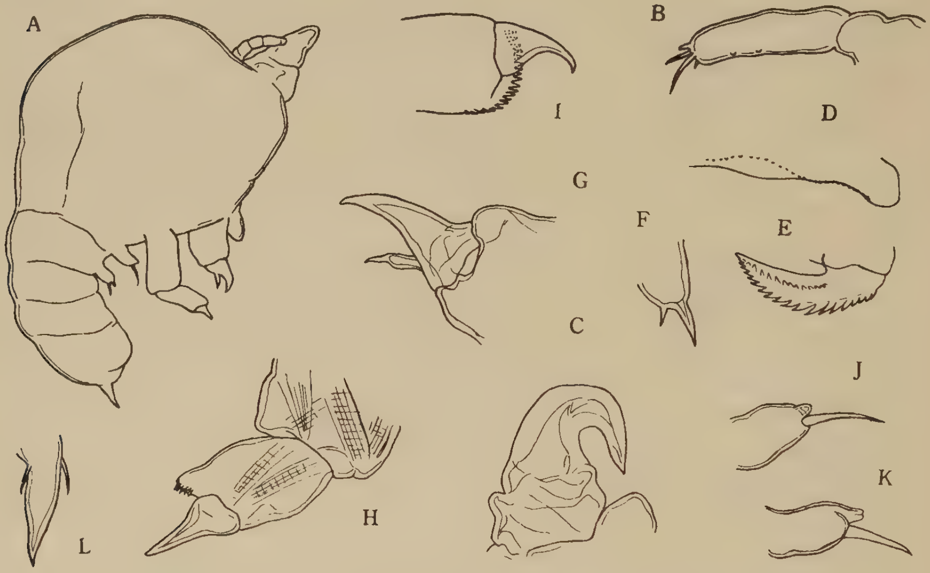

Fig. 22. *Acanthochondria bicornis* n. sp., male. A, lateral view; B, 1st antenna; C, 2nd antenna; D, labrum; E, mandible; F, maxilla; G, tip of 1st maxilliped; H, 2nd maxilliped; I, tip of same, viewed from other side; J, 1st leg; K, 2nd leg; L, caudal ramus. A ×160, B, D–L ×486, C ×83.

Remarks: It is rather unusual for *Acanthochondria* to possess a pair of processes on the head, since possession of these belongs to one of the peculiarities of *Chondracanthus*. In the latter, however, a number of processes are developed on the thorax as well. The present species is, so to speak, intermediate between the two genera in character, so that it may be optional to refer it to whichever of them. In so far as the species lacks the thoracic processes, it appears to me preferable to assign it to *Acanthochondria* rather than to *Chondracanthus*. In the general outline of the body, especially in the presence of cephalic barbs and the absence of thoracic processes, the new species resembles closely *Pseudochondracanthus diceraus* Wilson[20, 22], but is fundamentally different in having 2 pairs of legs. In Wilson's species, only a single pair is present and this is the reason for which I hesitate to place the new species in *Pseudochondracanthus*.

By the way, it may be well to add that, on account of the possession of 2 pairs of legs, *Pseudochondracanthus longitruncus* YAMAGUTI[23] cannot be retained in that genus but ought to be placed elsewhere.

Acanthochondria dilatata n. sp.

(Figs. 23 & 24)

Occurrence: On *Helicolenus dactylopterus* (DE LA ROCHE), taken off Kumano District, by Mr. K. SUZUKI, December 18, 1952. 3 females each accompanied with dwarf male, 1 of the former being selected as holotype. They bury their heads in tumors formed on soft parts of gill arches of host.

Female: Color whitish. Length from head to egg strings 7.15, same to abdomen 4.71, same to posterior processes of thorax 4.75, head 1.53×0.71, 1st thoracic segment, length 0.95, same, width in anterior region 0.52, same, in posterior region 0.67, 2nd segment 0.62×1.34, 3rd plus 4th segment 1.43×2.68, same, length including posterior process 1.58, genital segment 0.19×0.38, abdomen 0.21×0.17, egg tube 2.86.

Body consists of elongate slender anterior and inflated flabelliform posterior portions. Head more than twice longer than wide, columnar, tapering forwards only slightly and with oral appendages at posterior end of ventral face. First thoracic segment 2/3 as long as head, with anterior region prolonged into cylindrical neck which is slightly narrower and indistinctly bordered from head; posterior region a little but abruptly widening to form a trapezium which shows distinct hind border and bears legs on ventral surface. Second segment much wider but shorter than 1st, with width exceeding twice its own length, with round anterior shoulders, slightly narrowed backwards and lacking hind border. Third and 4th segments entirely coalesced to form a strongly expanded flabelliform segment which is wider than long and widest along caudal margin. Lateral margins evenly curved, converging forwards to meet those of preceding segment at right angles, and obtuse at caudo-lateral angles. Caudal margin widely round and carrying a pair of short narrow linguiform lobes just outside of abdomen, beyond which these scarcely extend. Genital segment hemispherical, abdomen ovoidal. Egg tubes empty.

First antennae short in relation to width, unjointed, tipped with a single rudimentary hair. Second antennae hook-like as usual, with terminal part slender, bluntly pointed and making a right angle with basal part. Blades of mandibles narrow, falciform, with denticles much feebler on anterior border than on posterior, becoming almost reduced near base. Maxillae roughly triangular, with 2 stout apical spines of unequal length and a small basal accessory lobe. Exopod of 1st maxillipeds short, straight, tapering to a blunt apex, and with a longitudinal surface rim in addition to relatively small number of marginal denticles; endopod

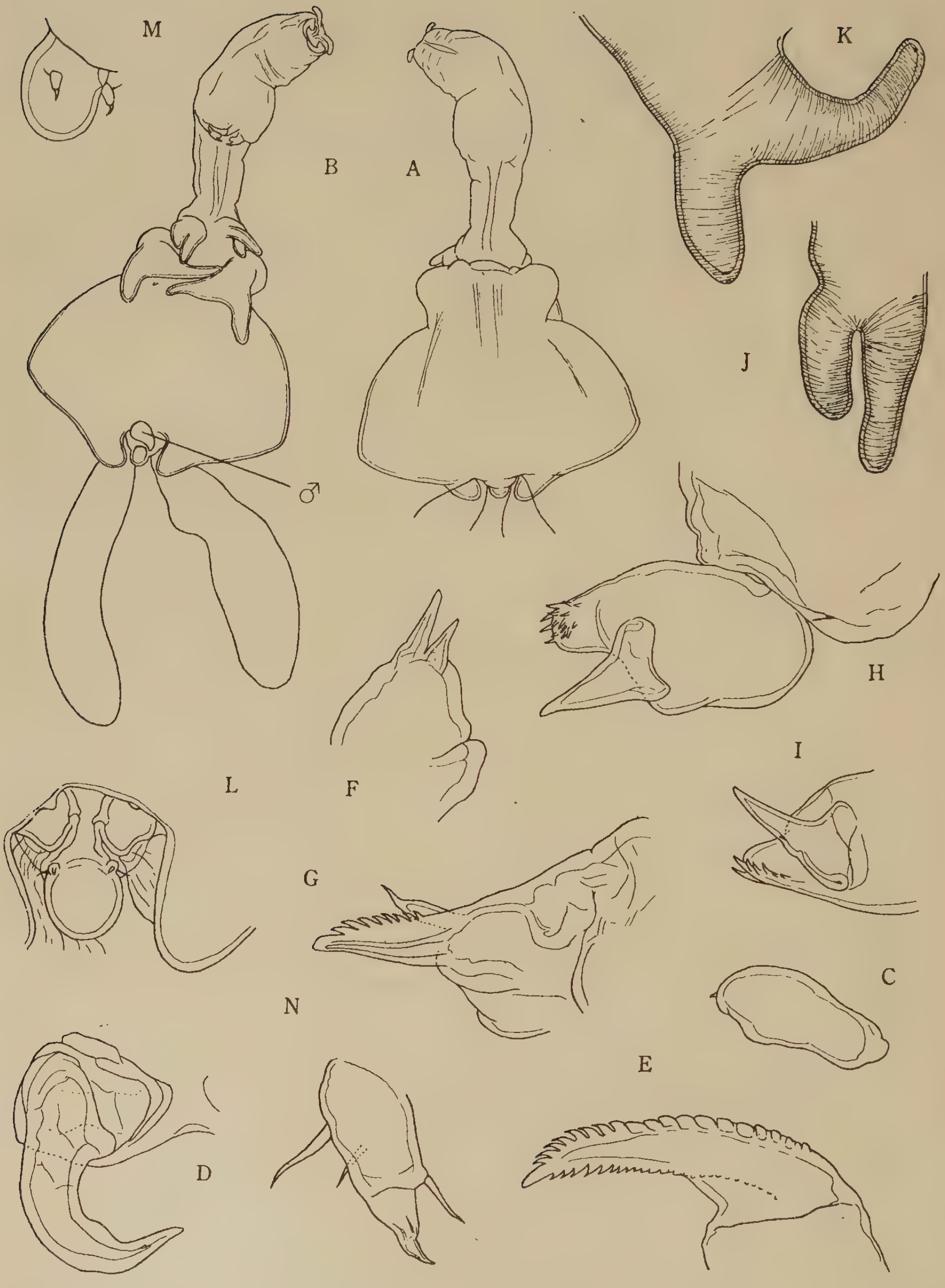

Fig. 23. *Acanthochondria dilatata* n. sp., female. A, dorsal view; B, ventral view; C, 1st antenna; D, 2nd antenna; E, mandible; F, maxilla; G, 1st maxilliped; H, 2nd maxilliped; I, same, inner view; J, 1st leg; K, 2nd leg; L, genital segment and abdomen, ventral view; M, same, ventro-lateral view; N, caudal ramus. A, B ×14, C ×120, D ×149, E–G ×486, H, I, N ×340, J, K ×41, L, M ×52.

rod-shaped, ending in spiniform tip. Terminal joint of 2nd maxillipeds nearly straight, acuminating to end, without accessory spine; inner distal angle of penultimate joint produced in a prominent truncate bulge, bearing bunch of spinules. Two pairs of legs unequal in size and shape. Anterior pair deeply bifurcate, with 2 branches parallel and blunt; outer branch shorter than inner and with a constriction close to base on outside. Branches of posterior pair also blunt, divergent from each other, making a right angle; outer member shorter, directing backwards and with a tiny basal spine instead of constriction, while inner member longer, slightly slenderer and projecting inwards. Surfaces of legs thickly covered with fine parallel rims encircling their axes transversely. Caudal rami 2-jointed; basal joint longer than apical joint, cylindrical, with a hair on inner side at end and 2 hairs on outer side about the middle, apical joints shorter, conical, sharply pointed, but somewhat crinkled.

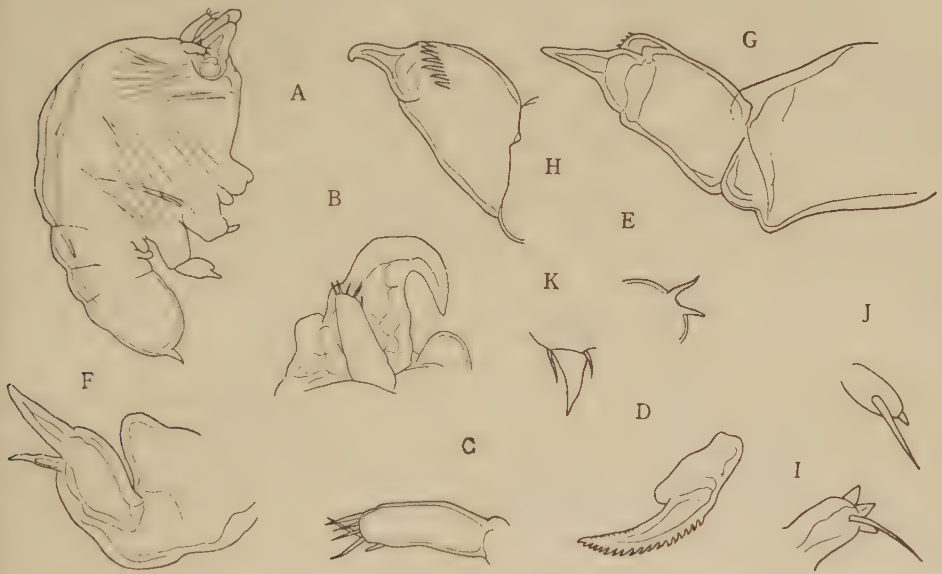

Fig. 24. *Acanthochondria dilatata* n. sp., male. A, lateral view; B, 1st and 2nd antennae in situ; C, 1st antenna; D, mandible; E, maxilla; F, 1st maxilliped; G, 2nd maxilliped; H, apical part of same, viewed from other side; I, 1st leg; J, 2nd leg; K, caudal ramus. A ×120, B ×340, C, I–K ×486, D–H ×595.

Male : Body not strongly crooked, pygmy, colorless and pellucid. Length 0.35, head, length 0.25, same, thickness 0.21, free segments, length on dorsal side 0.21, same, on ventral side 0.13, thickness 0.09.

Head ovate, more convex dorsally than ventrally, indistinct from 1st thoracic segment. Other segments hardly distinguishable from one another, lines of border remaining inconspicuous. Frontal plate low, semicircular. First antennae short, rod-shaped, neither curved, nor articulated, but tipped with a few hairs. Second

antennae hook-like, with terminal part of hook rapidly tapering to a sharp point; base with external bulge. Mandibles falcate, relatively slender, with denticles on anterior edge rather feeble and entirely reduced in its basal half. Maxillae crescent lobes with 2 subequal spinules. Exopod of 1st maxillipeds attenuating to blunt tip, nearly straight, and with entire borders; endopod slender, ending in a spine; 2nd joint of sympod oblong oval, while basal joint with inner distal bulge. Claw of 2nd maxillipeds curved only at its narrowed apex; penultimate joint with terminal bulge not prominent, crescentic, marginated with row of spinules. Two pairs of leg rudiments unequal in structure. First pair oblong, with 3 short conical spines at end and a long stiff hair just behind. Second pair narrower than 1st, ovate, with a short spine and a long hair. Caudal rami short, conical, with pointed tip, and with 2 basal setae, disposed 1 on dorsal and ventral sides respectively.

Remarks: Having anterior part prolonged into a cylinder whereas posterior part expanded in the shape of a fan, the body of the present species exhibits a rather singular appearance. As a result of elongation of the head the oral appendages are far removed from the antennae as in the Lernentominae[4,5,12]. But unlike in this subfamily, those appendages do not situate in the close vicinity of the legs but far apart again from these owing to the elongation of anterior portion of the 1st thoracic segment. Moreover, the head has no lateral expansions for anchoring the body within the host tissue, the possession of these being the most distinctive feature of this subfamily. It is evident from these facts that the present species cannot belong to the Lernentominae.

Leaving aside the elongation of the head, that of the 1st thoracic segment is not a rare occurrence in the Chondracanthinae, especially in *Acanthochondria*. The segment resulted from the union of the 3rd and 4th thoracic is expanded in a most unique fashion. But this character also does not go beyond the confine of this genus. Without doubt, *Acanthochondria* is the genus most appropriate for the new species.

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Biometrical Studies of a gobioid fish, *Chaeturichthys sciistius* JORDAN et SNYDER in the breeding season.

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The careful biometrical investigation of the seasonal changes of the gonad weight of *Fundulus* was carried out by MATHEWS, S. A. (1938). He concluded that the maximal mean value of the gonad weight appear in June and in a little while the mean value abruptly decreased, and the breeding season of this fish is maximal in a period extending from June to July. LECREN E. D. (1951) also observed the length-weight relationship and the seasonal cycle in gonad weight for the perch (*Perca fluviatilis* LINNE).

The present paper aims to present a biometrical account of some of the investigation on the biology of *Chaeturichthys sciistius* JORDAN et SNYDER collected in the breeding season.

Material:

The material used in this report consists of 1460 individuals, taken off Shiratsuka-Machi and Karasu-Machi, each situating in the neighbourhood of Tsu City, Mie Prefecture, by "Yokobiki" net from March to May in 1951.

The measurement of body length, body weight and gonad weight on which the present investigation is based were made according to sex distinction. The enumeration of number of eggs included in the right and left ovaries was made on some of the matured female fishes.

On account of our collection extending over two months, it is only rarely possible to compare the result of measurement of each date of collection, unless each fish group belongs to the same population. But it is unnecessary to require such exactness. Moreover, it is improbable that recognizable differences are kept in the same kind of fish inhabiting in the similar environment. If these differences are recognized, we shall not be able to continue our investigation. Therefore, during our research, we must assume that each fish group is referable to the same population.

Results obtained

I. Body length composition:

The mean value, standard deviation and probable error of body length on the distinction of each date of collection and sex are given in Table II. In the mean value of body length, the female is usually longer than the male. More-

over, the mean value and standard deviation on the distinction of each date of collection bear a close resemblance to each other for both sexes. Judging from this fact, it may be estimated that there is no differences between each body length composition for both sexes. The relationship between body length and body weight is not used by way of the determination of population, as the body weight is largely affected by the spawning. Again, considering the remarkable decrease of the body weight except gonad during this period as mentioned below, it seems to be regarded that the growth of the body length is largely kept under control.

II. Sex-ratio:

There is a considerable change in sex-ratio according to the advancement of the season as given in Table I. Generally speaking, the male is more numerous than the female during the period extending from March 6th to March 17th and

Table I. Number of material on the distinction of each date of collection and sex.

Date of Collection	Number of Material			Percentage	
	Male Fish	Female Fish	Total	Male Fish	Female Fish
March 6th	71	54	125	56.8%	43.2%
9th	38	93	131	39.0%	61.0%
10th	52	36	88	51.9%	40.9%
14th	52	44	96	54.1%	45.9%
15th	58	43	101	57.5%	42.5%
17th	60	39	99	60.6%	39.4%
25th	37	70	107	34.6%	65.4%
28th	75	191	226	28.3%	71.7%
April 3rd	59	107	166	35.6%	64.4%
16th	60	102	162	37.0%	63.0%
May 3rd	45	74	119	37.8%	62.2%
Total	607	853	1460	41.5%	58.5%

the male more numerous on and after March 25th. The above mentioned fact seems to have a close relation to the breeding season estimated the various observations. Namely, it comes to the conclusion that the female is more numerous in the maximal breeding season. Now it gives rise to discussion why the sex-ratio varies in each group which must be included in the same population owing to the body length composition respectively. There are several points of view in regard to this question as follows:

1. Some of the male may separate from their own population and migrate somewhere else.
2. The female inhabiting some other place may join to the population in question.

3. The female may be easily captured on account of exhaustion through the spawning.
4. The size of meshes may has a more unfortunate effect on the passing through of the female as compared with the case of the male.

But any valid conclusion can not be expected from our data.

III. Dairly change of body weight:

Body length is able to use as a means of determination of the population, as it is not affected on account of exhaustion through the spawning. But in the case of body weight, it varies greatly in accordance with the body length, and again it is obviously affected on account of exhaustion through the spawning. Therefore, the mean value of body weight on the distinction of each date of collection is originally insignificant as a means of determination of the population. However, if there is insignificant difference between each body length composition,

Table II. Distribution, Mean value, Standard deviation and Probable error of body length on the distinction of each date of collection.

(I). Male Fish

Body length \ Date	March								April		May	Total
	6	9	10	14	15	17	25	28	3	16	3	
3.9 cm										1		1
4.0												
4.1								1	1		1	3
4.2								2	4	1		7
4.3	1	1	3	2		2		3	6	4	2	24
4.4	2	3	3	2	3	3	1	6	9	9	2	43
4.5	3	3	4	2	3	3	3	11	4	10	9	55
4.6	11	7	5	5	8	7	8	12	8	8	6	85
4.7	8	2	12	5	11	13	7	13	10	10	7	98
4.8	14	8	7	8	17	12	7	14	6	7	2	102
4.9	10	5	11	8	8	7	6	6	3	3	3	70
5.0	5	3	2	7	3	6		4	2	1	4	37
5.1	3	1		4	3	1	2				2	16
5.2	2	3	1	4		1	2		2	1	1	17
5.3	3	1	2	1	1	2				1	2	13
5.4	1		2	2		1			2	3	1	12
5.5	2			2					2		1	7
5.6	3					1	1	1		1	1	8
5.7	3				1	1						5
5.8		1						2			1	4
Mean value	4.90	4.77	4.76	4.88	4.78	4.80	4.79	4.76	4.64	4.67	4.78	4.77
Probable error	0.027	0.032	0.024	0.026	0.019	0.024	0.026	0.025	0.032	0.028	0.037	0.085
Standard deviation	0.335	0.294	0.259	0.279	0.216	0.275	0.234	0.314	0.363	0.325	0.364	0.308

(II). Female Fish

Date / Body length	March								April		May	Total
	6	9	10	14	15	17	25	28	3	16	3	
4.3 cm									1			1
4.4		1						1	2		1	5
4.5				1				3		4		8
4.6	2			2	1		1	8	5	5	1	25
4.7	2	1	3	3	2	1	2	10	8	6	7	45
4.8	3	12	1	2	2	2	3	21	16	19	7	88
4.9	2	11	2	6	11	1	7	42	13	6	6	107
5.0	8	19	4	5	4	4	11	35	17	17	9	133
5.1	13	15	7	1	5	3	7	23	16	11	7	108
5.2	7	16	2	2	1	6	12	9	3	6	4	68
5.3	5	5	1	9	4	6	5	3	2	2	2	44
5.4	2	3	3	3	5	4	4	6	3	2	1	36
5.5	1	1		4		3	1	5	2	3	6	26
5.6		1	1			1	3	1	4	3	4	18
5.7	1	1					2	2	1	3	4	14
5.8	1	3			1		2	10	3	5	7	32
5.9	2	3	1	1		1	1	4		3	5	21
6.0	1	1	2	2	3	1	5	5	4	3	2	31
6.1			5	1		1	2	2	3			14
6.2	1			1	1	2		1	1	2	1	10
6.3	1				1	2	2		2			8
6.4	2		3		1				1			7
6.5				1	1							2
6.6			1			1						2
Mean value	5.24	5.12	5.22	5.23	5.25	5.41	5.28	5.08	5.11	5.12	5.26	5.18
Probable error	0.039	0.021	0.057	0.046	0.050	0.054	0.034	0.019	0.030	0.029	0.035	0.010
Standard deviation	0.428	0.304	0.510	0.449	0.487	0.502	0.418	0.390	0.460	0.440	0.440	0.417

it may be regarded that the mean value of body weight is statistically significant. The mean value and standard deviation of body weight on the distinction of each date of collection and sex are given in Table III. In the male, the mean value of body weight shows a maximum about March 14th and it decreases remarkably on and after March 25th, while in the female, it shows a maximum about a period extending from March 10th to 14th, thereafter, gradually decreasing, and indicates a minimum about April 3rd. Furthermore, the standard deviation of body weight shows a maximum about a period extending from March 10th to 15th in the female, but a remarkable change is not recognized in the male. Of course, the number of individuals is not enough to give credence to the value of standard deviation, but the remarkable variation recognized in female may be

regarded as an indication of some of change of body weight due to the maturation of gonad and the spawning.

IV. Dairly change of gonad weight:

The frequency distribution, mean value, and standard deviation of gonad weight grouped by date and sex are given in Table IV. In the male, the mean value for each group indicates a gradual decrease according to the advancement of the season, while in the female, the former indicates a rather complicated change, that is, the maximum appears on 10th March and thereafter the gradual decrease of the mean value according to the advancement of the season are seen until April 3rd, appearing the minimum mean value. In any case, it may be regarded that the spawning process arises obviously during the period of the gradual decrease. Then, in the female, the frequencies of gonad weight seem to have a mode at 0.6–0.7 gram. during the period extending from 6th to 9th in March, but these suddenly disparse, having a maximal standard deviation in March 10th. In this connection it should be mentioned that the gonad weight is increased by the maturation on the one hand, and is decreased by the spawning on the other hand. Again, in March 25th, the frequencies are capable to subdivide into two groups, that is, in the one group these are distributed at 0.1–0.4 gram. and in the other group at 0.8–1.6 gram. This subdivision is recognized, too, in April 16th. But the dermacation between two groups becomes unrecognizable gradually with advance of the season on and after April 3rd. The

Table III. Distribution, Mean value and Standard deviation of body weight on the distinction of each date of collection.

(I). Male Fish

Date / Body weight	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
1.2 g								2		1	
1.5		1			2			4	13	6	3
1.8	10	5	3	1		5		21	18	15	20
2.1	13	19	6	2	4	14	2	26	20	22	15
2.4	15	7	12	8	14	20	11	19	3	10	3
2.7	13	2	13	13	13	11	12	1	3	3	3
3.0	5	4	10	10	15	5	10		1	1	
3.3	6		5	10	7	3	1		1	1	1
3.6	3		1	1	2	2		1		1	
3.9	2			4	1		1				
4.2			2	1				1			
4.5				2							
Mean value	2.476	2.225	2.700	2.994	2.747	2.470	2.700	2.088	1.983	2.090	2.013
Standard deviation	0.585	0.353	0.513	0.568	0.462	0.426	0.345	0.427	0.392	0.424	0.343

(II). Female Fish

Body weight \ Date	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
1.2 g										1	
1.5	1							1	2		
1.8		1						16	24	12	5
2.1	3	7	2	1		1		24	27	22	7
2.4	4	20	3	1	3	1	8	37	23	19	14
2.7	10	38	4	6	5	3	13	43	9	21	12
3.0	11	11	2	5	8	9	18	32	3	7	7
3.3	12	7	9	4	10	5	6	12	2	5	11
3.6	5	2	1	8	5	4	6	11	7	5	8
3.9	1	3	1	4	5	8	5	4	1	4	7
4.2		2	4	6		3	3	6	4	4	2
4.5	1	1	1	3	1	2	4	1	4		1
4.8	4	1		1	1	2	1	3	1	2	
5.1			3		3		3	1			
5.4	1		2	1		1	1				
5.7											
6.0	1		2	1			2				
6.3			2	1	1						
6.6											
6.9				2	1						
Mean value	3.222	2.793	3.816	3.825	3.558	3.547	3.400	2.752	2.492	2.635	2.919
Standard deviation	0.845	0.527	1.115	1.098	0.960	0.702	0.876	0.636	0.774	0.900	0.876

appearance of the group, distributed at 0.1–0.4 gram., may be regarded as an indication of the decrease of gonad weight due to the spawning. Moreover, in addition to the above-mentioned fact, the hardly recognizable subdivision in March 17th obviously showed that the spawning arises remarkably during the period extending from 17th to 25th in March. This period surely corresponds to the period that the female are more numerous than the male. The appearance of the frequencies distributed at neither group on and after March 25th is looked upon as the increase of gonad weight which has been once decreased on account of the spawning. Now there arise a question whether it is proper or improper to arrange these data of gonad weight at the same time, because these data are obtained from the measurement of both non-spawned and spawned individuals on and after March 25th. It is insignificant to arrange these heterogeneous data, as in spite of the mean value on March 25th indicating 0.470 gram., there is no corresponding individual in the gonand weight class of 0.5 gram. . Therefore at the time of consideration, it must be taken statistics in respective cases. It may be regarded that individuals having the gonad weigning less than 0.4

gram. correspond spawned fishes, and more than 0.4 gram. correspond non-spawned fishes. Again, it is apparently insignificant to take together data on the period extending from April 3rd to May 3rd, because these comprise individuals indicating the secondary increase of gonad weight after the spawning in addition to both non-spawned and spawned individuals. Subsequently, it must be become a subject of discussion whether the spawning of individual is carried out during the short period or the considerable period. It may be concluded that the spawning is carried out during the short period on account of the absence of individuals having gonad indicated an intermediate weight on March 25th.

To sum up the above-mentioned explanation, it may be concluded that in the case of male, the gonad gradually decreases in weight on and after March 6th according to the advancement of season but in the case of female, the gonad weight about 0.7 gram. on an average in the beginning of March, then gradually increasing in weight with the advance of season until about the middle of March when it reaches about 0.9 gram. on an average, and then it abruptly decreases in weight until 0.25 gram. on an average in the end of March on account of the spawning, but since then it gradually increases in weight.

V. Change of body weight except the gonad:

The body weight except the gonad on an average indicates the maximum

Table IV. Distribution, Mean value and Standard deviation of gonand weight on the distinction of each date of collection.

(I). Male Fish

Date / Body weight	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
0.00 g											
0.05		1							1	1	
0.10				1				1	2	8	10
0.15		1	1	6	7	7	6	11	14	21	25
0.20	9	1	4	15	14	18	7	30	20	16	10
0.25	25	12	14	12	19	20	13	20	15	8	
0.30	16	11	18	13	8	9	7	8	6	4	
0.35	14	10	10	3	8	5	3	4		2	
0.40	3	1	4	1	2	1	1	1	1		
0.45	2	1	1								
0.50	1										
0.55	1										
0.60											
0.65				1							
Mean value	0.294	0.291	0.296	0.250	0.252	0.242	0.246	0.226	0.209	0.185	0.150
Standard deviation	0.0715	0.0685	0.0595	0.0835	0.0655	0.0590	0.0630	0.0575	0.0595	0.0333	0.0333

(II). Female Fish

Date / Gonad weight	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
0.1 g						3	1	6	2	3	2
0.2	1			2	2	1	33	56	36	27	14
0.3	2		1	2	2	4	15	20	25	12	4
0.4	7	14	6	6	4	3	4	1	18	11	8
0.5	13	33	3	9	7	2		1	8	4	6
0.6	9	17	4	3	5	7		4	11	11	12
0.7	8	16	3	7	4	5		10	1	15	11
0.8	3	6	2	1	4	4	2	13	2	10	11
0.9	2	4	5	3	3		3	21	2	6	5
1.0	1	1	2	3	6	3		28			1
1.1	2		1	2		2	4	16		2	
1.2	2	1		2	3	1	1	10	2	1	
1.3	1	1	1	2		3	2	1			
1.4			2	1	1		1	2			
1.5	1		3			1	2	1			
1.6	1						2	1			
1.7			1								
1.8	1			1							
1.9					1						
2.0			2								
2.1											
2.2					1						
Mean value	0.702	0.596	0.894	0.736	0.765	0.682	0.470	0.640	0.367	0.563	0.536
Standard deviation	0.306	0.169	0.463	0.350	0.404	0.385					
Mean value for gonad weight indicating less than 0.4 g.							0.242	0.219	0.273	0.258	0.264
Mean value for gonad weight indicating more than 0.4 g.							1.182	0.962	0.659	0.896	0.700

about March 14th, since then decreasing gradually in each sex of fish, but it gradually increases about May 3rd in the female only. Therefore, it may be appreciated that the body weight, the gonad weight and the body weight except the gonad are changed parallel each other according to the advancement of seasons.

VI. Number of Ovarian eggs:

The enumerations of number of eggs included in the right and left ovaries were separately performed on 75 female individuals. At the time of enumeration, eggs which are to be spawned in this year were enumerated, but non-developed eggs were eliminated from the enumeration.

Table V. Mean value of body weight except the gonad on the distinction of each date of collection.

W........Body weight
G........Gonad weight
W—G........Body weight except the gonad

(I). Male Fish

Date	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
W	2.476	2.225	2.700	2.994	2.747	2.470	2.700	2.088	1.983	2.090	2.013
G	0.294	0.291	0.296	0.250	0.252	0.242	0.246	0.226	0.209	0.185	0.150
W—G	2.182	1.934	2.403	2.744	2.495	2.228	2.454	1.862	1.774	1.905	1.863

(II). Female Fish

Date	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
W	3.222	2.793	3.816	3.825	3.558	3.547	3.400	2.752	2.492	2.635	2.919
G	0.702	0.596	0.894	0.736	0.765	0.682	0.470	0.640	0.367	0.563	0.536
W—G	2.520	2.197	2.922	3.089	2.793	2.865	2.930	2.112	2.125	2.072	2.383

The average, maximum and minimum of eggs are as follows; namely the first is 3446, the second 6302 and the third 2114. Besides the standard deviation of average number is 788.

The difference in numbers of eggs between the right and left lobe of ovary is recognized and the right lobe has generally more numerous eggs than the left lobe. Only 12 individuals among 75 individuals have more numerous eggs in their left lobe of ovary. While the average difference in numbers of eggs between the two lobes is 583 in the case of having more numerous eggs in the right lobe, it is 209 in the case of opposite. The reason for such phenomena

Table VI. Mean value of body weight grouped by the class of body length on the distinction of each date of collection.

(I). Male Fish

Body length \ Date	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
3.9 cm										1.170	
4.2	1.640	1.620	1.937	2.060		2.020		1.605	1.618	1.582	1.490
4.5	1.987	1.966	2.338	2.376	2.333	2.118	2.471	1.848	1.851	1.920	1.786
4.8	2.349	2.235	2.711	2.890	2.741	2.420	2.673	2.172	1.976	2.147	1.993
5.1	2.565	2.647	3.307	3.257	3.332	2.911	3.048	2.385	2.558	2.455	2.327
5.4	3.440	2.990	3.798	4.214	3.440	3.163			2.718	3.048	2.408
5.7	3.303	2.820			3.970	3.000	3.840	3.250		2.690	2.595

(II). Female Fish

Body length \ Date	March								April		May
	6	9	10	14	15	17	25	28	6	16	3
4.2 cm									2.900		
4.5	2.280	1.800		2.487	2.570		2.720	2.073	1.707	2.061	1.925
4.8	2.673	2.355	2.480	2.975	2.942	2.398	2.525	2.440	1.957	2.177	2.250
5.1	2.979	2.707	3.167	3.469	3.162	3.165	2.740	2.704	2.353	2.521	2.698
5.4	3.124	3.118	3.890	3.944	3.702	3.599	3.541	3.092	3.563	2.821	3.371
5.7	3.425	3.838	4.290		4.770	4.550	4.034	3.705	3.125	3.357	3.625
6.0	5.047	4.245	5.004	5.788	4.463	4.270	4.741	3.958	3.921	4.074	3.560
6.3	4.993		6.247	6.100	5.457	4.635	5.480	4.570	4.528	4.180	4.420
6.6			7.300	6.900	7.000	3.670					

can not be traced from our data, but it may be regarded that the difference in number of eggs between the two lobes of ovary is a sort of various asymmetries and bilateral variations in fishes.

Table VII. Mean value of gonad weight grouped by the class of body length on the distinction of each date of collection.

(I). Male Fish

Body length \ Date	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
3.9 cm										0.120	
4.2	0.240	0.270	0.270	0.135		0.235		0.200	0.184	0.188	0.157
4.5	0.266	0.310	0.288	0.257	0.251	0.244	0.246	0.288	0.218	0.198	0.152
4.8	0.318	0.302	0.311	0.269	0.257	0.248	0.255	0.242	0.222	0.201	0.158
5.1	0.303	0.313	0.320	0.293	0.275	0.259	0.228	0.238	0.188	0.155	0.169
5.4	0.290	0.170	0.400	0.188	0.170	0.213			0.255	0.148	0.158
5.7	0.263	0.030			0.270	0.260	0.390	0.180		0.090	0.145

(II). Female Fish

Body length \ Date	March								April		May
	6	9	10	14	15	17	25	28	3	16	3
4.2 cm									0.470		
4.5	0.560	0.350		0.347	0.470		0.850	0.448	0.199	0.550	0.225
4.8	0.561	0.490	0.423	0.573	0.590	0.395	0.244	0.632	0.241	0.484	0.374
5.1	0.585	0.579	0.662	0.600	0.669	0.728	0.405	0.666	0.363	0.448	0.571
5.4	0.589	0.741	0.900	0.718	0.822	0.850	0.444	0.728	0.449	0.486	0.705
5.7	0.280	0.644	1.050		1.020	1.250	0.511	0.532	0.550	0.442	0.571
6.0	1.607	1.028	1.131	1.193	0.847	0.270	0.739	0.597	0.553	0.609	0.467
6.3	1.183		1.663	0.370	1.123	0.395	0.910	0.290	0.768	0.255	1.020
6.6			1.950	1.750	2.150	0.300					

VII. Mean value of body weight and gonad weight grouped by the class of body length.

On account of the recognition of an insignificant difference in the body length compositions on the distinction of each date of collection, it seemed to be unnecessary to group the body weight and the gonad weight according to their class of body length. But it becomes to be necessary to consider this matter in the present case for a significant difference is generally recognized in most cases.

The mean value of body weight and gonad weight grouped by their class of body length on the distinction of each date of collection and sex are given in Tables VI and VII respectively. From the investigations of these tables, it may be concluded that the trends which have previously been described are occurred in the present case. Accordingly, the above-mentioned discussion on the body weight and gonad weight can be applied to the present case.

Summary

(1) The present paper aims to present a biometrical account of some of the investigations on the biology *Chaeturichthys sciistius* JORDAN et SNYDER taken off Shiratsuka-Machi and Karasu-Machi, each situating in the neighbourbood of Tsu City, Mie Prefecture during the period extending from March 6th to May 3rd in 1951.

(2) Upon the investigation of body compositions on the distinction of each date of collection and sex, mean values and standard deviation are approximate each other. Furthermore, in the mean value of body length, the female is always longer than the male.

(3) There is a considerable change in sex-ratio according to the advancement of the season. Generally speaking, the male is more numerous during the period extending from March 6th to 17th and the female more numerous on and after March 25th. Namely, it comes to the conclusion that the female is more numerous in the maximal breeding season.

(4) The dairly change of body weight on the distinction of sex were investigated. In the male, the mean value shows a maximum about 14th and decreases remarkably on and after March 25th, while in the female, it shows a maximum about the period extending from March 10th to 14th, thereafter gradually decreasing, and indicates a minimum about April 3rd. Furthermore, the standard deviation of body weight shows a maximum about the period extending from March 10th to 15th in the female but shows unremarkable change in the male.

(5) The dairly change of gonad weight on the distinction of sex were investigated. In the male, the mean value of gonad weight indicates a gradual decrease according to the advancement of season, while in the female, the former indicates a rather complicated change, that is, the maximum appears on March

10th, thereafter the gradual decrease of the mean value are seen until April 3rd, and again the gradual increase are seen on and after April 3rd.

(6) The body weight except the gonad is obviously changed parallel with the body weight and gonad weight according to the advancement of season.

(7) The average, maximum and minimum numbers of ovarian eggs are as follows; namely the first is 3446, the second 6302 and the third 2114. Besides the standard deviation of average number of eggs is 788.

(8) The mean value of the body weight and gonad weight grouped by their class of body length on the distinction of each date of collection and sex are investigated.

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Report of Faculty of Fisheries,
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Vol. 2, No. 1, Oct. 30, 1955.

The species of the genus Gelidium from Japan and its vicinity (I)

(With 9 Plates and 10 Text-figures)

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The systematic study of the genus *Gelidium* in Japan was made by the late Dr. Okamura. At first he described 13 species of it in "On Gelid. and Pterocl. of Japan" (Jour. Imp. Fish. Inst. vol. 29, No. 2) in 1934. In the next year he described again 6 species of it in "Taiwan-san Tengusarui ni Tsuite" (in Japanese) (Japan Assoc. Advanc. Sci. vol. 10, No. 2, 1935) and furthermore, two years later described 16 species in "Nippon Kaisō Shi" (1936). It was summed up generally with his fine illustrations. Only a few phycologists but him in Japan reported them respectively.

About this time, these several years the writer has been studying the classification and development of the *Gelidium* which is the most important material of agar in Japan, re-scrutinized the studies hitherto made, and at present enumerated about 24 species from Japan including 1 new species, 10 species new to Japan and recognized many unsuitable points in the studies hitherto made. The writer, therefore, wants to correct them as noted below.

Hitherto, in this genus outward shape of frond and branch was acknowledged as the characteristics on systematics. The writer thinks, however, that number of cortical layers and distribution of rhizoids in the inward structure as well as the outward shape are also important criteria for discrimination. But it cannot be denied that there is even now some doubtful points in this difficult and complexous study. Some modifications may be made in the future studies, yet the writer will be very glad if the present study makes something clear in the determination of the species and contributes some knowledge to the study of classification.

In this study the writer examined many foreign specimens through the good offices of Prof. Y. Yamada, and many specimens and papers in the Hokkaidō University as well. Furthermore, Dr. E. Yale Dawson in the Allan Hancock Foundation, University of Southern California and Mr. Kung-chu Fan in the Taiwan Fisheries Research Institute sent him many specimens. Prof. M. Honda in Tōkyō University and Prof. J. Tokida, Dr. S. Segawa, Prof. T. Tanaka, Dr. Y. Nakamura and Mr. Y. Hasegawa related to Hokkaidō University gave him hearty kindnesses. Here the writer wishes to express the very best thanks to

them. Also the writer must acknowledge that the pecuniary support was given to the present study partially by the scientific research promotion fund of the Ministry of Education.

Gelidium johnstonii SETCHELL et GARDNER

Plate 5 and Text-fig. 1.

New Mar. Alg. from the Gulf of Calif. (1924), p. 742, pls. 72, 73 and 46, a; DAWSON, Mar. Alg., Gulf of Calif. (Allan Hancock Pac. Exped., vol. 3, No. 10, 1944), p. 260; Id., Guide to Litera. Distri. of Mar. Alg. Pac. Coast of North Amer. (Memoirs, South. Calif. Acad. of Sci. vol. 3, Part 1, 1946), p. 59; Id., Recon. Alg. Mar. (Revis. Soci. Mexi. Histo. Natu. IX, 1948), p. 237; Id., Upwell. Assoc. Veget. (Sears Found. Journ. Mar. Res. 1951), p. 52; Id., Investi. Reci. Sobre Alg. Mari. (Revis. Soci. Mexi. Histo. Natu. XIII, 1953), p. 128; SEGI, Spec. of Gelid. from Japan and its vicin. (1) (in Japanese) (Bulle. of Jap. Soc. of Phyc. vol. 2, No. 1, 1954), pp. 15–17, Figs. 1–2.

Syn. *G. kintaroi* FAN (non YAMADA), Gelid. and Pterocl. of Taiwan (Labor. Biol. Rep., No. 2, 1951), p. 12, pl. 4, fig. 1; text-fig. 7.

Japanese name: *Tsuyakusa* (nov.).

Hab.: Gotō, Fukue and Sakiyama (Y. YAMADA, T. TANAKA), Kitauomemura and Sone (Minami-matsuura Fishery Guild), Hizen Prov.; Daili (T. TANAKA), Keelung (T. TANAKA, FAN) and Tansui (FAN), Formosa.

Distri.: Pacific coast of North America.

The frond of this species is robust, linear and complanate. It is about 5.5–8 cm. in height and 2–3 cm. in width, 310–370μ in thickness in the central part of the main axis. It is tapering abruptly at the base, gradually narrowing upwards. It branches in irregular, patent and rather pinnate manner upwards. The branches are recurved, flexuose and densely issuing clavate ultimate branchlets in an opposite manner. The branchlets are twice or thrice forked at their apices. The medullary cells are scattered and round or elliptical in shape. They are large measuring from about $16 \times 18\mu$ (smallest) to $26 \times 28\mu$ (largest) in size. The rhizoidal filaments are aggregated forming a mass in the outer layer of the central tissue. They are scattered in the central part, being about 6μ in diameter. The cortical cells are forming (2)–3–(4) layers. They are lumpish in shape and 4–7μ in size. The tetrasporangia are dotted numerously in the apices of ultimate branchlets and divided in cruciate manner. The cystocarps arise one or two beneath the apex of ultimate branchlets. They are elliptical, rather large, longitudinally arranged, swollen on both sides and bilocular. The sori are formed on both sides of the central axis. The pericarps are swollen, as if covering sori. They are provided with paraphyses attached to the central part of

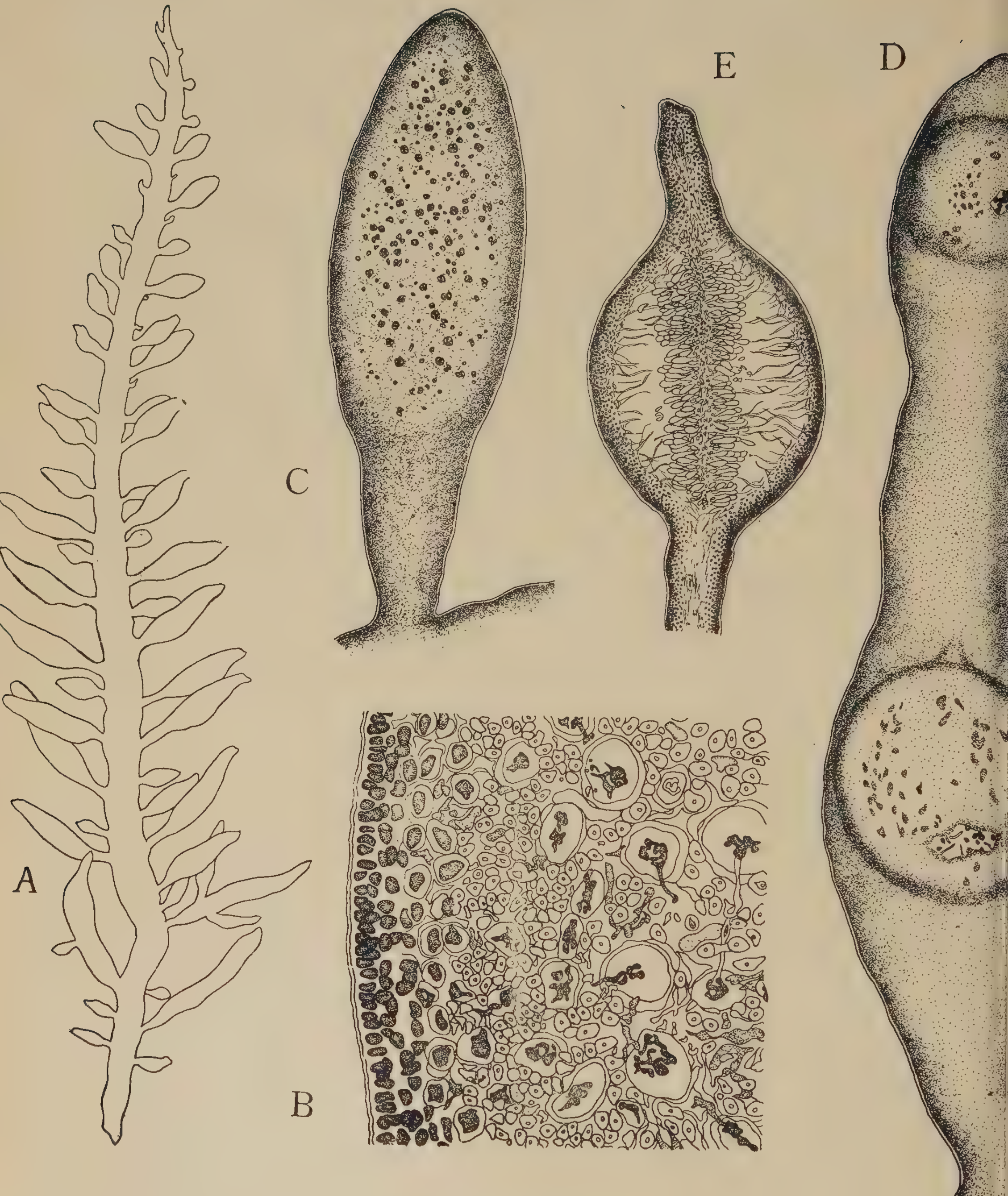

Fig. 1. *Gelidium johnstonii* SETCHELL et GARDNER
A. Branch. ×ca. 7; B. Transverse section of the frond. ×400; C. Stichidium. ×54; D. Ultimate branchlet bearing cystocarps. ×54; E. Longitudinal section of the cystocarp. ×54.

sori connecting pericarps and sori. In the central part of pericarp there is a small unprominent carpostoma. The frond is rather rigid cartilaginous in the substance and shows dark purplish red in colour.

The present species is characterized by possessing glossy colour, ultimate pinnulae issuing densely from main branch and especially aggregated ultimate pinnulae having cystocarps, flatness of frond, cortex composed of 2–4 layers, etc.

Abundant specimens from Gotō, Daili and Keelung are all belong to this species. The former are tetrasporic plants and coincide with the original description and pl. 73, and the American specimens (Gulf of Calif. coll. det. E. Y. Dawson No. 1941, 7170). The latter are cystocarpic fronds and issuing densely ultimate pinnulae with cystocarps from main branches. In the inner structure they are composed of two cortical layers and show a similar distribution of rhizoids as noted above in the description. These specimens also agree well in general with the original description and pl. 72 and American specimens (Gulf of Calif. coll. det. E. Y. Dawson, No. 471, 532, 7043). Furthermore, in these American specimens they are also composed of 2 cortical layers. The state of distribution of rhizoids is similar with that of the specimens noted above.

Mr. Fan described *G. kintaroi* in his above cited paper, but his species is to be identified with *G. johnstonii* as noted in p. 128 of this paper.

Gelidium kintaroi (OKAMURA) YAMADA

Plate 6 and Text-fig. 2.

Notes on some Japan. Alg. IX (Sci. Pap. Inst. Alg. Res. Hokkaidō Imp. Univ. 2, 1941), p. 201; Segi, Spec. of Gelid. from Japan and its vicin. (1) (in Japanese) (Bulle. of Jap. Soc. of Phyc. vol. 2, No. 1, 1954), pp. 17–19, Figs. 3–4.

Syn. *G. clavatum* Okamura, On Gelid. and Pterocl. of Japan (Jour. Imp. Fish. Inst. vol. 29, No. 2, 1934), p. 47, pls. 28, 32, figs. 4–6; Id., Nippon Kaisō Shi (1936), p. 465.

Japanese name: *Shakugatabuto*

Hab.: Amoy, Formosa.

Dr. Okamura established this species as *G. clavatum* Okamura, basing on the specimens from Bōkotō and Amoy. He indicated the former as 1 and the latter as 2 in pl. 28 in his paper cited above. The writer, however, came to the conclusion that the two should be classified into different species, according to his careful examination on two type specimens. At present it is a good chance for him to solve and explain the confusion.

(a) On the specimen from Bōkotō

This specimen is a female plant, glossy and rather rigid cartilaginous. The main branch is patent, densely issuing pinnulae. In the inner structure it

is composed of 2 cortical layers and the rhizoidal filament is aggregated in the outer-layer of central tissue forming a mass. Evidently this specimen should be identified with *G. johnstonii* SETCHELL et GARDNER noted above and coincide with the Californian specimens of this species (Gulf of Calif. coll. det. E. Y. DAWSON No. 471, 532).

Mr. FAN in Formosa described that the plant growing from Kōkaten over to Sekimon (indicates Rōbai specimen in l. c. pl. 4. fig. 1) is similar to Bōkotō specimen determined by OKAMURA. The writer also agrees with him. Accordingly, these Formosan specimens should be identified with *G. johnstonii*, not with *G. kintaroi*. The Taiwan specimen from Tansui, Keelung (signed as *G. latifolium* (GREVILLE) BORNET ?) containing tetrasporic and cystocarpic fronds are dwarf, compressed and about 4–5.2 cm. high. Among them the tetrasporic frond expands to semi-globose outline, issuing the branches oppositely. The branches are densely covered with short pinnulae. The cystocarpic one is provided with branches in irregular or opposite manner, issuing pinnulae sparsely or densely.

Furthermore, he separated the Tansui specimen from all species of Formosan *Gelidium* and noted that they bear resemblance to *G. corneum* LMX. v. *latifolium* GREVILLE or *G. latifolium* (GREVILLE) BORNET. He sent also the specimens from Tansui and Keelung to the writer. After examination, they are found by the writer not to be the species of GREVILLE or BORNET cited above, but identified with *G. johnstonii*. Besides the descriptions and figures of *G. latifolium* (GREVILLE, Alg. Brit., 1830, Tab. XV; HARVEY, Phyc. Brit., vol. III, 1846–51, pl. 53 (191), fig. 3; FELDMANN et HAMEL, Gélidiales, 1936, p. 245, fig. 25) the writer was able to examine the specimens from Europe (Banyuls, medit., MAZOYER; Fort de la coté Se Serum). The frond of *G. latifolium* branches out into only two or three main branches forming a broad clavate-lanceolate shape and issuing spinulate pinnulae from branchlets regularly in an opposite manner, while the frond of *G. johnstonii* branches more and the branches are rather elongate, not broad as the former and the ultimate branchlets of it are rather aggregated forming a mass. In addition, in the inner structure the former is composed of 2 cortical layers, while the latter of 2–4 cortical ones.

(b) On the specimen from Amoy

The frond of this specimen is flattened, branching off in a pinnate manner. The branch is out irregularly in alternate or opposite manner, very patent, blunt at the apex. The ultimate branchlets are arising a few in an opposite manner on the apex of clavate branchlet. The plant is membranaceous in the substance, thinner at the apex and somewhat reddish in colour. In the lower part it is rigid and reddish. As one of the important characters, in the inner structure it is composed of one layer's cortical cells (forming rectangles, about $7 \times 4\mu$ in size) and the rhizoidal filament (about 5μ in diam.) is aggregated between the cortical

Fig. 2. *Gelidium kintaroi* (OKAMURA) YAMADA
A. Branch. ×ca. 3; B. Transverse section of the frond. ×400.

layer and central part. The cells of central part are scattered and 25×18–13×10 μ. It also bears resemblance to the descriptions and figures of *G. corneum* LMX. var. *pinnatum* GREVILLE (TURNER, Fuci, 1819, vol. IV, p. 146, fig. d; HARVEY, Phyc. Brit., vol. III, 1846–51, pl. 53, Fig. 1; GREVILLE, Alg. Brit., 1830, Tab. XV) and foreign specimens (Herb. LENORMAND, communicat. et Herbario Lungduno-Batavo, Génes). Thus it seems to be related to *G. corneum*, but seems to the writer more reasonable to refer this specimen to *G. kintaroi* than to *G. corneum* in the following several points. The cortical layer is composed of one layer completely, the rhizoidal filaments are scattered between the central part and cortical layer, the frond has no percurrent axis and the branch is patent in the apex. As for only one specimen, however, there still remains some doubt. Furthermore, Mr. FAN noted that *Gelidium* sp. showing on l. c. p. 13, pl. 4, fig. 2 resembles to Amoy specimen. Judging from the description and figure given by him (l. c. p. 15, fig. 8), however, it is composed of 2 cortical cells, rigid and stout and two times as thick as the frond (500μ, while in Amoy specimen about 224μ). Considering these points, it seems to be different from Amoy one.

In conclusion, the writer now thinks it is reasonable to refer Bōkotō specimen to *G. johnstonii* SETCHELL et GARDNER and Amoy specimen to *G. kintaroi* (OKAMURA) YAMADA respectively.

Gelidium polystichum GARDNER

Plate 7 and Text-fig. 3.

New Spec. of Gelid. on the Pac. Coast of North Amer. (Univ. Calif. Publ. Bot., vol. 13, 1927), p. 276, pl. 41; DAWSON, Guide to Litera. Distri. of Mar. Alg. Pac. Coast of North Amer. (Memoirs, South. Calif. Acad. of Sci. vol. 3, Part 1, 1946), p. 59; Id., Mar. Flo. Chan. Isl. (1949), p. 24; Id., Investi. Reci. Sobre Alg. Mari. (Revis. Soci. Mexi. Histo. Natu. XIII, 1953), pp. 128–129.

Japanese name: *Yezotengusa* (nov.).

Hab.: Yunohama, Okujiri Isl., Hokkaidō (Y. HASEGAWA).

Distri.: Pacific coast of North America.

The frond is caespitose, rigid, linear, complanate and standing from fibrous root. It is about 6.7 cm. high, 1 mm. wide and branched in polystichous, pinnate manner. The branches arise from somewhat upper part of the base, sometimes forming several orders, occur primarily along the margines of the flattened frond with wide axils (45–90°), issuing in opposite or alternate manner. They are straight or slightly flexuose, narrow at the base, issuing spinous ultimate branchlets in opposite manner. The branchlets are rather straight, linear and blunt in the apex. The medullary cells are elliptical and situated between the cortical layer and central part, large, about 27×20μ diam. They are gradually smaller towards cortical layer and central part, having thick cell membrane.

Fig. 3. *Gelidium polystichum* GARDNER

A. Branch. ×ca. 7; B. Stichidium. ×54; C. Transverse section of the frond. ×400.

The rhizoidal filaments are aggregated between the cortex and the medulla, scattered in the medulla, about 5μ diam. The cortical layer is composed of 1–(2) layers and rather elongated elliptical cells. The tetrasporangia are aggregated on the upper, swollen part of short, ultimate, pastulate branchlets.

This species is characterized by possessing the complanate section having branches of all orders arising normally from the flattened surfaces as noted by original author. The degree of polystichy is variable as noted by DAWSON in l. c.

This plant has been hitherto tentatively referred to *Pterocladia tenuis* OKAMURA. Judging from the description and plate given by GARDNER, it is identified with the species cited above, though the frond is not so high and the upper branch is not so aggregated as the plate of GARDNER. The rhizoidal filaments are observed numerously between the cortex and the central part or under the subcortical layer, though Dr. DAWSON noted "in the inner cortex" in l. c. This plant was collected in Okujiri Isl., Hokkaidō, at the beginning of October, though Dr. DAWSON noted "This species is apparently ephemeral, disappearing before summer, for it has not been found in summer collections from the same area". In North latitude Okujiri Isl. is situated north of Santa Catalina Isl.

Being compared with some foreign specimens, the Yunohama plants agree well to one Californian specimen (Santa Catalina Isl., Calif. det. coll. E. Y. DAWSON, No. 5720). The frond of No. 5720 attains to 7–9 cm., but No. 5640 to 30 cm.

Furthermore, this plant resembles the other foreign specimen (*G. arbuscule* BOERGESEN, Algae Marine Canarienses det. Leg. F. B. Insula: Teneripe Orotava No. 3253, which is almost unramified, sparsely and not so finely branched, 5–7.5 cm. high), especially in the simple ramification and similar size. But it shows more resemblance to *G. polystichum* which branches finely.

This species has not been previously described in Japan.

Gelidium purpurascens GARDNER

Plates 8–13 and Text-figs. 4–10.

New Spec. of Gelid. on the Pac. Coast of North Amer. (Univ. Calif. Publ. Bot., vol. 13, 1927), p. 275, pls. 38, 39; KYLIN, Calif. Rhodo. (1941), p. 7; SMITH, Mar. Alg. Monte. Penin. (1943), p. 197, pl. 44, fig. 6; DAWSON, Guide to Litera. Distri. of Mar. Alg. Pac. Coast of North Amer. (Memoirs, South. Calif. Acad. of Sci. vol. 3, Part 1, 1946), p. 59; Id., Investi. Reci. Sobre Alg. Mari. (Revis. Soci. Mexi. Histo. Natu. XIII, 1953), p. 129.

Japanese name: *Murasakibuto* (nov.).

Hab.: Yonezaki-mura, Akazaki-mura, Otomo-mura and Matsuzaki-mura, Rikuchū Prov.; Mogusa, Hokkaidō (ONO).

Distri.: Central California; Mexico.

The frond is stout, linear, complanate and standing from fibrous root. It is 12.5–15 cm. in height, 1.5–3 mm. in width in the lower part, gradually narrowing upwards and branching 7–8 times in a pinnate manner. The branches are distantly and irregularly branching from somewhat upper part of the base,

Fig. 4. *Gelidum purpurascens* GARDNER f. *elongatum* SEGI (Specimen from Yonezaki-mura)
A. Branch. ×ca. 3.5; B. Stichidium. × 54; C. Transverse section of the frond. ×400.

sometimes fastigiated, in the lower part with axils of about 45°. It is elongated, straight or somewhat flexuose, constricted at the base, issuing many short, spinous ultimate branchlets in opposite or irregular manner on both sides. The medullary cells are scattered, round or elliptical, about 12×20–$17 \times 30\mu$, having thick cell membrane. The rhizoidal filament is aggregated at the inner side of the cortex, about 4.5–6μ diam. The cortical layer is composed of 2–(3) layers, forming rather square cells. The tetrasporangia are aggregated elliptically at the top of pastulate ultimate branchlets and divided in a cruciate manner. The frond is rather rigid cartilaginous in the substance and dark purplish red.

Being used rich material collected by the writer, the present species shows various kinds of types, but is divided into two main forms: f. *elongatum* and f. *pinnatum*.

f. *elongatum* f. nov.

Frondes elongatae planae, inferiore latae, superiore tenues sparsae, irregulariter fastigiatae ramosae.

Frond elongate, flattened, broad below, narrow above, sparsely, irregularly and fastigiately branched upwards.

The description noted above is basing on the specimen from Yonezaki-mura. In the specimen from Akazaki-mura the frond is more elongated than that of Yonezaki-mura, about 21.5–23 cm. high and has an indistinctive axis. The frond branches off with round axils in the lower, but the branchlets in the upper arise numerously with narrow axils in opposite or irregular manner. The rhizoidal filaments are aggregated between the cortical layer and the centre. The other specimen attains to about 11 cm. high and expands in a semiglobose outline, issuing many spinous ultimate branchlets in almost opposite manner. The branches are elongated, flexuose and not so fastigiated.

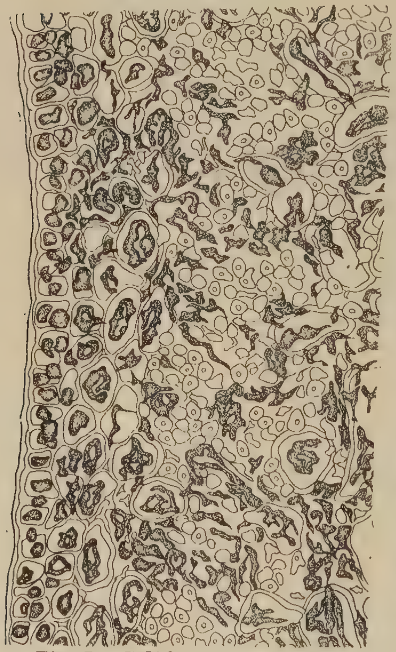

Fig. 5. *Gelidium purpurascens* GARDNER f. *elongatum* SEGI (Specimen from Akazaki-mura) Transverse section of the frond. ×400.

Fig. 6. *Gelidium purpurascens* GARDNER f. *pinnatum* SEGI (Specimen from Akazaki-mura) Transverse section of the frond. ×400.

f. *pinnatum* f. nov.

Frondes solitariae, lineares, compressae, multae pinnatim ramosae, semiglobosae expansae.

Frond solitary, linear, compressed, pinnately much branched, expanded into a semi-globose outline.

The present form from Akazaki-mura has only one axis and branches in a pinnate manner. It attains to about 9 cm. high and expands in a semiglobose outline, issuing many branchlets in irregular manner. The rhizoidal filaments are aggregated between the cortical layer and the centre forming a mass and are scattered numerously towards the centre.

Dr. Okamura showed two type specimens on pl. 24 in his "On Gelid. and Pterocl. of Japan" (1934) calling *Gelidium subfastigiatum* Okamura. According to the writer's examination of original specimens signed as type in the Hokkaidō University, however, the specimen of the upper (Oshoro) seems to be identified with *G. subfastigiatum* and the lower (Kesenuma, Otomo-mura, No. 63) with *G. purpurascens*. The frond of the latter is nude below, densely branched above in a pinnate manner. It is composed of 1–(2) cortical layers. The cortical cells are rather small in size. The rhizoidal filaments arise numerously between the cortex and the central part. The tetrasporangia are numerously formed inwards the cortical and subcortical layers as figured (fig. 7). This agrees well with Smith's pl. 44, f. 6, Gardner's pl. 38 and the foreign specimen (Moss Beach, June 1929, La Jolla). In these American plants the frond is composed of 1–(2) cortical layers. The rhizoidal filaments are scattered from inner side of the subcortical layer to the central part. It resembles *G. amansii*, but not branching off at the base, expands itself upwards.

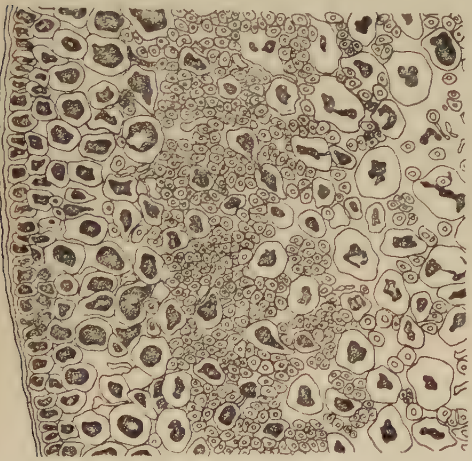

Fig. 7. *Gelidium purpurascens* GARDNER f. *pinnatum* SEGI. Transverse section of the frond. ×400. From Okamura's specimen (as *G. fastigiatum* OKAMURA) shown in Okamura's l.c. Pl. 24 (lower).

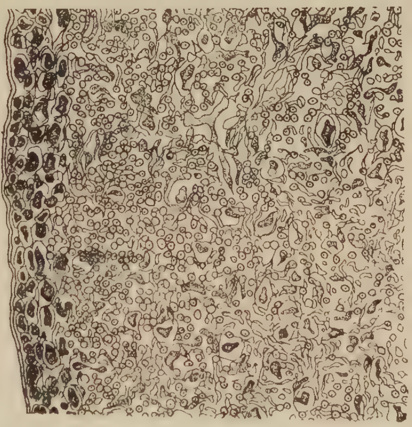

Fig. 8. *Gelidium purpurascens* GARDNER f. *elongatum* SEGI (Specimen from Otomo-mura) Transverse section of the frond. ×200.

Besides, the writer examined the following three specimens owned by Dr. Okamura and found them all belong to this species.

(1) The specimen from Otomo-mura, Kesengun shown in photograph

(signed as *G. irregularis*) accords with the plate 39 of GARDNER's paper (1927) and belongs to f. *elongatum.* It is about 8.5 cm. high, about 1.8 mm. wide and expands in semi-globose outline. The frond is broad, flattened, less divided in the lower part, finely divided in a pinnate manner upwards. It is composed of 1–(2) cortical layers. The cortical cells are rather irregular in shape. The rhizoidal filaments are aggregated numerously at the inner side of the subcortical layer and are scattered to the medullary part.

(2) The specimen from Matsuzaki-mura, Kesengun, signed as *G. subfastigiatum* is also belonging to *G. purpurascens* f. *elongatum.* It represents a linear form, 21.5 cm. high, standing from fibrous root, broad near the base, about 2 mm. wide and rather flattened. The frond is sparsely branched. The branches are bearing numerous short branchlets which are irregularly or alter-

Fig. 9. *Gelidium purpurascens* GARDNER f. *elongatum* SEGI (Specimen from Matsuzaki-mura) Transverse section of the frond. ×200.

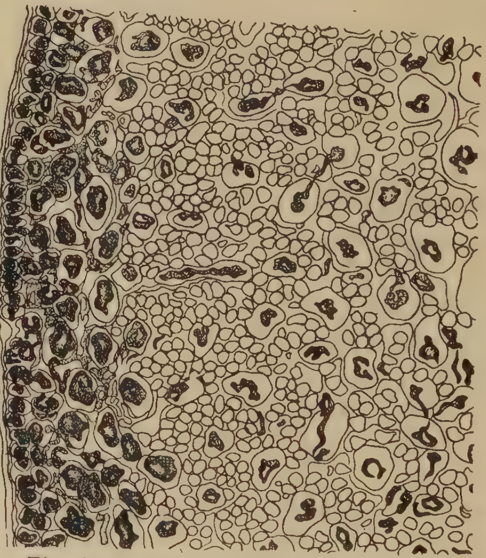

Fig. 10. *Gelidium purpurascens* GARDNER f. *pinnatum* SEGI (Specimen from Mogusa) Transverse section of the frond. ×400.

nately or oppositely placed. The frond is composed of 1–(2) cortical layers. The cortical cells are small in size and rather irregular in shape. The rhizoidal filaments are aggregated numerously between the cortex and the central part. This specimen resembles *G. setchellii* (GARDNER, l. c., p. 275, pl. 40), but it may better be identified to be one form of *G. purpurascens.*

(3) The specimen from Mogusa is hitherto identified with *G. subfastigiatum,* but it is desirable to be placed with *G. purpurascens* f. *pinnatum.* It is about 14 cm. high and expands in semiglobose outline. The frond is divided into several segments and in the upper part issuing many branches and forming a

Plate 5. *Gelidium johnstonii* SETCHELL et GARDNER
(Specimen from Gotō) ×ca. 1.

Plate 6. *Gelidium kintaroi* (OKAMURA) YAMADA
Type specimen from Amoy) × ca. 3/5.

Plate 7. *Gelidium polystichum* GARDNER
(Specimen from Yunohana) ×ca. 1.

Plate 8. *Gelidium purpurascens* GARDNER
f. *elongatum* SEGI
(Specimen from Yonezaki-mura) ×ca. 5/6.

Plate 10. *Gelidium purpurascens* GARDNER
f. *pinnatum* SEGI
(Specimen from Akazaki-mura) ×ca. 1.

Plate 11. *Gelidium purpurascens* GARDNER
f. *elongatum* SEGI
(Specimen from Otomo-mura) ×ca. 7/9.

Plate 12. *Gelidium purpurascens* GARDNER
f. *elongatum* SEGI
(Specimen from Matsuzaki-mura) ×ca. 3/4.

Plate 13. *Gelidium purpurascens* GARDNER
f. *pinnatum* SEGI
(Specimen from Mogusa) ×ca. 4/7.

bundle. The rhizoidal filaments are densely aggregated from inner side of the subcortical layer to the central part. The tetrasporangia are several placed in the cortical and subcortical layers and divided in cruciate or tripartite manner.

This species is a new addition to the marine aigal flora of Japan.

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Retinal and Optic Nerve Response of the Compound Eye of Spiny Lobster, *Panulirus japonicus* (VON SIEBOLD)

(*With 5 Figures*)

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Light stimulation evokes electric responses in retinas. Although many attempts have been made, there still remains much to be explained on the mechanism of the discharge of nerve impulses in the afferent fibers from sensory receptors.

The possible conclusion about the visual excitation so far can be briefly outlined as follows: decomposition of light-sensitive substances of the retina, when stimulated, is the first process of retinal response. The slow changes of electrical potential across the retina, recorded after a short latent period, will show a correlation to the generation of nervous activity.

Since the vertebrate retina, with its complicated cross-connexion of nervous

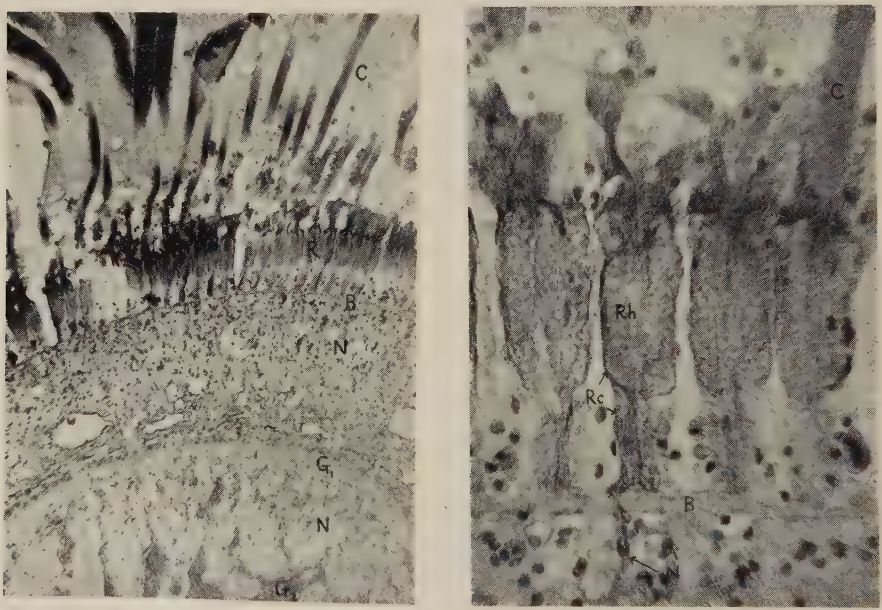

Fig. 1. Photomicrograph of the spiny lobster eye showing retinal cells and ganglions. C, crystal cone; R, retina; B, basal membrane; N, nerve fiber; G_1, 1st ganglion; G_2, 2nd ganglion; Rh, rhabdomere; Rc, retinula cell.

pathways, is a complicated originator of electrical potentials, the information of the relation between the retinal response and the nerve response is expected to be difficult. It is of importance, therefore, to try it out with primitively organized eye. Hartline's works on the eye of the horseshoe crab are of great importance.

The present paper is concerned in a comparative physiological study of the magnitude of retinal potential and nerve response in the compound eye of *Panulirus japonicus*.

Technique and procedure

Preparation:

The soft carapace of the dorso-anterior of the head is opened and the optic nerve is readily found. It can be easily dissected free of the surrounding tissue and severed at a convenient length. The eye, with an eye stalk, is then loosened from the animal and removed with its attached length of nerve. It was placed on the Ringer soaked cotten pad. As electrodes were used silver-silver chloride wires. Now and then the preparation got wet with Ringer solution. In order to lead off from the front of the whole eye a small hole was cut in the chitinous cornea through which the point of the electrode could be inserted.

Apparatus:

The light of 6 V. 50 c.p. lamp is concentrated from the optical system with a condensing lens, a slit and a hand-operated shutter. The terminal focus has a diameter of 6 mm. The maximal illuminance of a surface placed in the focus is 250 lux; initial adapting illuminance 10000 lux. The chamber in which the preparation is situated is electrically shielded and light being tight.

The recording of the retinal action potentials was carried out with a Yokogawa-made electromagnetic-type oscillograph in conjunction with a directly coupled amplifier designed by Dr. Hanaoka, and modified by the author. At maximum sensitivity 30 microvolts being applied to the input of the first stage, which produces a 16 mm deflection of the oscillograph beam at the camera. The amplifier used for recording the nerve impulses is a four stage R.C. coupled one.

Results

The typical electroretinogram (ERG) of the whole eye is shown in Fig. 2, and in this case the active electrode is placed on subcornea and the other on the back of eye.

After a latent period of approximately 0.06 sec. illumination causes increased negativity of the cornea relatively to the back of the eye. The response is a

Fig. 2. Retinal action potential. Deflection upward indicates increasing negativity of the cornea with respect to back of the eye. Time in 1/10 sec.

Fig. 3. Retinal action potentials recorded with various electrode combination. The numbers 1–3 show the different loci used for the active electrode, whereas 4 is the constant locus of the reference electrode. Time in 1/10 sec.

simple monophasic deflection, rising sharply to a maximum, and subsiding more slowly downward to the original base line. The negativity is sustained during the illumination. There is no "off-effect" at the cessation of light, but only a slight depression of the curve appears after some latent time. The ERG of *Panulirus* resembles that of *Limulus* and also of cray-fish.

Both of the electrograms obtained from whole eye and from bisected eye are entirely the same in size and shape, if the electrode is placed on the same position in each case. And the position of the active electrode on the corneal surface or on the inner margin of the bisected cornea, seems to make no difference in the electrical process. These accounts are explained by the fact that the receptors arrange themselves side by side with an acute regularity from a standpoint of electric field and they electrically respond to light at a time in the same manner as suggested by HANAOKA.

When the active electrode is successively placed on each point from cornea to the center across the retina (Fig. 3), the electric current changes its direction in the neural side of retina. This evidences that the slow potential originates from the receptor themselves.

The records in Fig. 4 were taken from the experiment with the active electrode placed on subcornea and the other on post-ganglionic optic nerve.

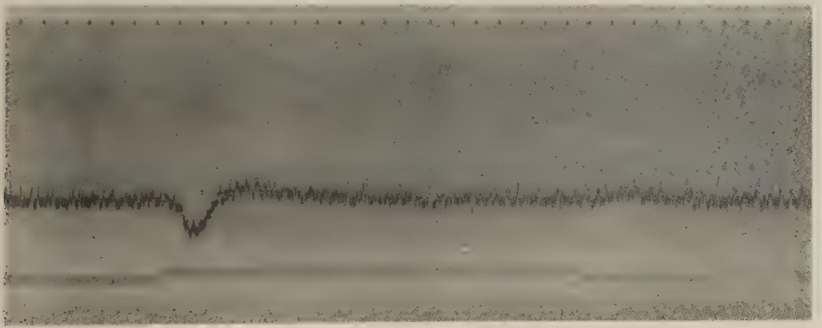

Fig. 4. Spontaneous spike activity. Explanation in text.

Here a spontaneous spike activity is visible on the curve; in this preparation, however, no increase in nerve impulses to the stimulation could be recorded. As the examples of typical responses of the post-ganglionic whole optic nerve, the author offers the records reproduced in Fig. 5. The nerve always shows spontaneous discharges even in the stationary state. In the preparation with no or less spontaneous activity, there could not be found any burst of nerve discharges responding towards the light stimulation. The stimulation is followed by the impulses as large as 0.17 mv. in average after a short latent period. There is an irregular variation of potential, usually consisted of the first group of the burst of nerve discharge and the second group following a silent period (Fig. 5, rec. A), whereas in Record B the silent period can not be so clearly

observed as in Record A. The discharge continues as long as the light is kept put in on the eye, and the impulses show a slow fall of the frequency in the discharge. The off-response is not clear as compared with the on-response; in most preparations the off-discharge is not seen or can not be distinguished from

A

B

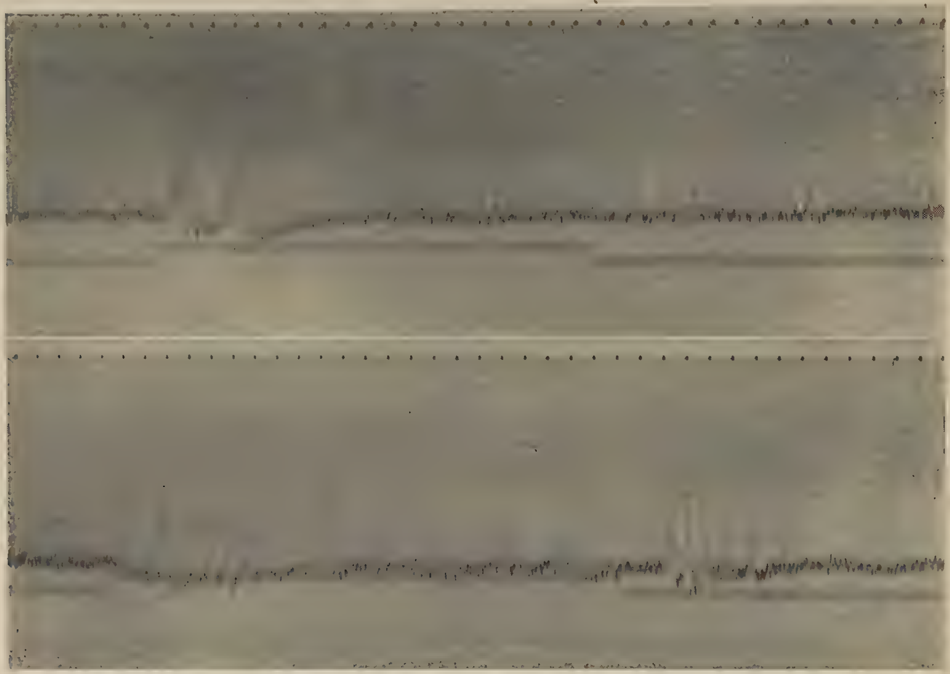

Fig. 5. Action potentials from the whole optic nerve. Time in 1/10 sec.

the spontaneous activity. But in Fig. 5, rec. B, the off-discharge is clearly recognized. As is demonstrated in Fig. 5 rec. A, the accerelation of spontaneous impulses is generally seen after the cessation of light; the frequency of the spontaneous discharge increasing.

Discussion

The retinal action potential in *Panulirus* closely resembles what is found in the similar preparations of cray-fish, and the off-effect can not be recorded as in *Limulus* eye. The aim of the author's work has been at the magnitude of discharge of nerve impulses. As was demonstrated by HARTLINE, the record of off-discharge and the spontaneous impulses in the stationary state can not be got in *Limulus* eye. There are the respects in the picture of activity of the nerve obtained by the author, which differs from what suggested in HARTLINE's work.

The author has found spontaneous activity to be a very common phenomenon in the preparations. This difference seems to be due to the structure of eye and to the difference in method. In *Limulus* eye there are neither ganglion cells nor synapses. The author supposes that the spontaneous activities recorded

in his preparations may originate from the ganglion itself on the ground of its leading off from the post-ganglionic nerve.

It is interesting that the rising phase of the retinal action potential is agreed in phase with the first burst of nerve activity. And this synchronism in *Limulus* has been demonstrated by HARTLINE. This evidence shows that these initially discharged photoreceptor units may be adaptable fairly rapidly.

The interpretation of the relationship between the retinal potentials and the discharge of nerve impulses must be left for further experiments. Considering the magnitude of nerve activity, a complexity seems to be retained in the visual process of the eye of *Panulirus*, in spite of the simplicity of retinal action potential. The occurrence of off-discharge may suggest the presence of the inhibitory process as suggested by GRANIT.

Summary

Action potentials have been taken into record from the isolated compound eye and the optic nerve of the *Panulirus japonicus*. The receptors in the eye under illumination react with a monophasic potential, rising sharply to a maximum, in relation to the back of the eye the front of the visual elements being negative. No off-effect in the electroretinogram has ever been seen. In the post-ganglionic whole optic nerve the response to light consists both of the irregular burst of nerve impulses responding to the light stimulation and the spontaneous discharges. The increases in the frequency of the spontaneous activity have been observed after the cessation of light. The discharge in the whole nerve begins after a short latent period at the first burst which is approximately synchronous with the rising phase of the on-effect of retinal action potential. Following a short pause in the discharge the second burst can be recognized. The frequency finally falls, which is maintained for the duration of illumination. The off-discharge can be recorded in spite of the simplicity of the retinal action potential. These accounts suggest the presence of the complex process, such as inhibitory one, in the visual excitation of the eye of *Panulirus japonicus*.

The author's many and hearty thanks are due to Prof. N.Y. KAWAMOTO, D. Sc. of our laboratory, and Dr. T. HANAOKA, Prof. of Nara Women's Univ., for their helpful criticism and advices, and especially to Mrs. S. AOKI for her much useful discussion.

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Electrical Studies on the Compound Eye of *Eriocheir japonicus* DE HAAN

(*With 6 Figures*)

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Introduction

The complexity of the response of the vertebrate retina to light soon led physiologists to simpler eyes in order to study the isolated responses of the primary sensory cells. The work of HARTLINE represents a beginning in this direction. The application of the microelectrode technique to the compound eye will certainly to much in assigning the components of the electroretinogram (ERG) to the responsible structure. The widely accepted theory on the analysis of ERG so far is based on GRANIT's component analysis. Recently, OTTOSON and SVAETICHIN have demonstrated an interesting evidence that the polyphasic ERG of the frog originates in the receptors alone; positive on and off, and negative on-and-off responses, appear to originate in rods and cones respectively.

The following conception of the compound eye which ERG has emerged the photoreceptors upon illumination become more negative at their distal ends (extracellular recording). The receptor negativity is sustained during illumination unless the optic ganglion responds, after the initial negativity, with a potential opposite to that produced by the receptors. Receptor and ganglion potentials may then sum themselves to produce such features as on-and-off-peaks in the ERG and a return to the base line potential during illumination.

BERNHARD analysed the ERG of water-beetle. According to BERNHARD the receptor layer of the *Dytiscus marginalis* eye is responsible for two negative ERG components, while the optic ganglion contributes a third which is opposed in sign to the other two.

The present paper deals with the results of the electrical studies on the compound eye of *Eriocheir japonicus*.

Preparation and Method and Apparatus

Whole excised compound eyes with eye stalk were used; if not, the removal of the optic ganglion damage the receptor unless the microelectrode technique is employed. This preparation was made under a dim red lamp after suitable dark adaptation. Recordings of the eye were made with non-polarizable macroelectrode

(Ag–AgCl electrodes). The active electrode placed on cornea and the other on the back of eye. Recording of the action potentials was carried out with a Yokogawa-made electromagnetic-type oscillograph in conjunction with a directly coupled amplifier designed by Dr. T. HANAOKA, and modified by the author. At maximum sensitivity, 30 microvolts applied to the input of the first stage produced 1.6 mm deflection of the oscillograph beam at the camera. The cornea was oriented in the terminal focus of the optical system which included a 6V. lamp as a source of white light, a condensing lens system, a slit, a hand-operated shutter. The terminal focus had a diameter of 6 mm. The maximal illuminance of a surface placed in the focus was 150 lux. Initial adapting illuminance was 10000 lux.

Results

Wave form and polarity

Records on the intact animal are almost similar to that of isolated eye, except the occurrence of small irregular variations of potential originating muscle and gill movement. Typical ERG of isolated dark-adapted eye is reproduced in Fig. 1. In all responses of isolated eye the lead from the illuminated eye records initial negativity after a short latent period (about 0.02 sec.), then the negativity is sustained during illumination. When the active electrode is placed on the subcornea, slight negative deflection is recorded now and then after the short positive deflection immediately following the negative-on-effect. During the sustained illumination the base line potential is regained. The off-effect is more complex and diphasic deflection is observed. After a latent period of approximately 0.04–0.07 sec. cessation of light causes a small negative response following a positive deflection. This complex off-effect can not be seen in the compound eye of *Limulus* and also *Panulirus japonicus*; their responses are monophasic, rising sharply to a maximum, and subsiding more slowly towards the original base line. Some insect ERG's consists of complex on-effect, a sustained base line or near base line potential during illumination, and complex off-effect. The complex off-effect recorded in the eye of *Eriocheir* may be attributed to the contribution of the ganglionic component.

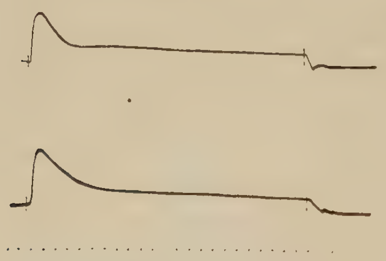

Fig. 1. Retinal action potentials. Upper record is potential recorded leading by subcornea and back of eye. Lower record, leading by surface of cornea and back of eye. Light signal is across curve. Time in 1/10 sec.

Dark adaptation

Light adaptation may be described as a loss in sensitivity. This is a re-

versible process, and sensitivity is fully recovered (dark adaptation) if the eye is kept for a sufficiently long time in darkness. The course of the dark adaptation has been investigated quantitatively by many experimenters in the compound eye of other animals. The purpose of this experiment is to deal with the variation of wave form under the condition of adaptation of the eye.

When returned to darkness after the exposure of several minutes (about 10 min.) to a bright light (1000 lux), the eye of *Eriocheir* shows only a feeble response for the first minute or two (light-adapted eye), but as time goes on its response to the same stimulus becomes stronger, until it finally reaches the maximum value as it can have for that stimulus. The records of Fig. 2 repre-

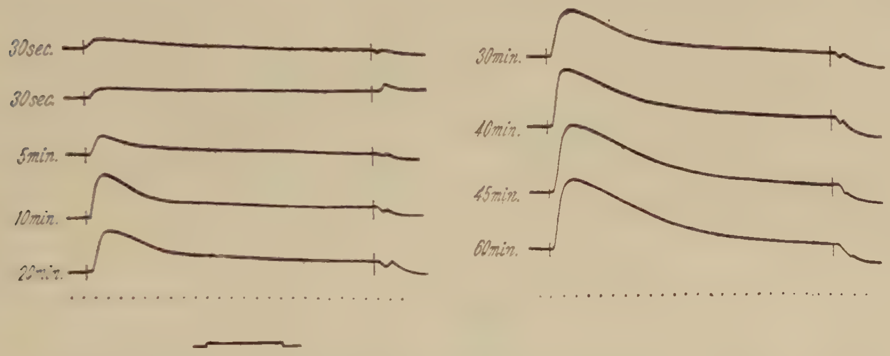

Fig. 2. Retinal action potentials at various times in dark. Calibration to 30μV. Time in 1/10 sec.

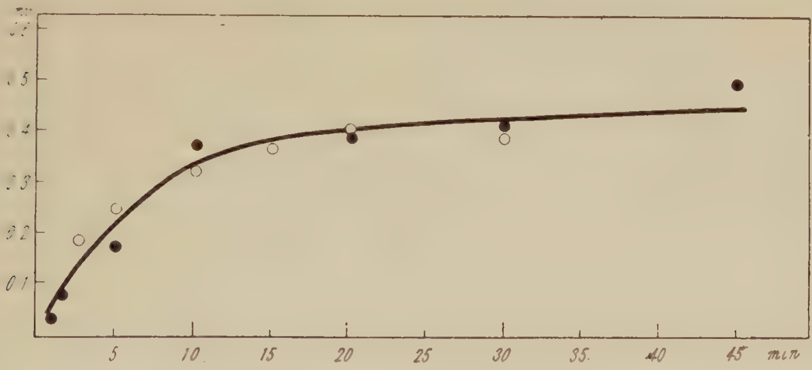

Fig. 3. The course of dark adaptation. The filled and open circle represent potentials for respectively about 2 and 1/30 sec. illumination. Time in dark, horizontal. Height of response, vertical.

sent a typical experiment of the progressive change of retinal response during dark adaptation. The course of the process is shown in Fig. 3. It is observed that the response elicited by a stimulus of constant energy increases rapidly during the first few minutes in the dark, and then more slowly, approaching towards a limiting value asymptotically. As is shown in Fig. 2, the response of

light-adapted eye differs from those of dark-adapted eye: the lower rate of rise in on-effect and slower negativity sustained during the illumination following the on-effect. In the experiments illustrated in Fig. 4, a short exposure of 1/30 sec. is chosen. The response of dark-adapted eye shows a positive deflection following the initial negative deflection. This positive deflection may be not a pure off-effect. The off-effect is more complex and not of the monophasic type; there it consists of a positive deflection, a spike-like negative effect after a short latent period (0.04–0.07 sec.). Most frequently after the prolonged dark adaptation, is observed the spike-like negative effect disappearing. In few experiments in which animals were adapted to light, the positive deflection could not be seen; the off-response was only a negative deflection. (Fig. 2).

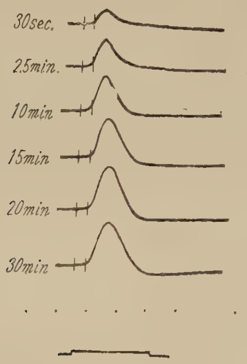

Fig. 4. Retinal action potentials in response to brief illumination of the eye at various times in the dark. Calibration to 30 μV. Time in 1/10 sec.

Discussion

The electrical changes recorded from the optic mechanism are influenced in their magnitude and complexity by the location of the electrodes. In the experiments of the compound eye of *Panulirus* under the same experimental condition, the complex off-effect, the upward peak at cessation of illumination reported in *Eriocheir*, could not be seen. When the light is turned out the negativity first drops quickly to a certain level, followed by a slow descent towards the base line. In the isolated compound eye of *Dytiscus*, there can be observed no negative off-deflections. BERNHARD concluded that the negative off-peak led off from the optic ganglion in his experiments with cocainized ganglions. It may be due to the difference in the nature of structured units that the complex off-effect is seen in the eye of *Eriocheir*, not in *Panulirus* under the same experimental condition. GRANIT has point out that, unless a particular care is taken in placing the electrodes, the recorded waves represent the algebraic sum of the potential changes generated in the retina, optic ganglion, and optic nerve. The complex wave pattern of the invertebrate ERG is generally interpreted as a summation of several components.

It is interesting to ascertain whether the negative action potential during the illumination in the eye of *Eriocheir* is homogeneous or whether it consists of components with different function or significance. As was shown in Fig. 2, after the prolonged dark adaptation the visual response rapidly reaches large values and rapidly falls immediately after initial rising phase. But if the eye is

stimulated with the same intensity after a few minutes in the dark the response looks quite different. Its rising phase is slower and the amplitude lower, and if the illumination is kept up for a long while the potential diminishes very slowly; no rapid falling phase can be seen. The positive deflection of dark-adapted eye recorded in the experiments with a short flash stimulation may perhaps correspond to the rapid falling component. Considering these facts, it may possibly be concluded that in the *Eriocheir* ERG there may be more than two monophasic processes of the same electrical sign, as was concluded by BERNHARD.

Of these components, the two are illustrated in Fig. 5; line S represents the component which is largely responsible for the shape of the light-adapted eye ERG and R the component, fast one, dominated in the dark-adapted ERG.

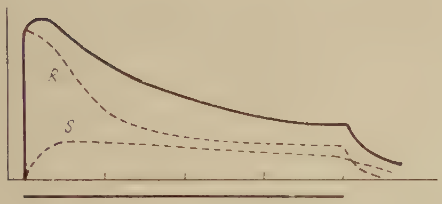

Fig. 5. Analysis of the erectroretinogram of the isolated compound eye. Lower line indicates a duration of stimulus.

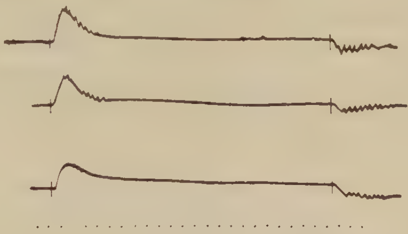

Fig. 6. Retinal action potentials five minutes after the administration of ethylalcohol. Time in 1/10 sec.

The visual response is suppressed by 10% ethylalcohol (Fig. 6), yet, no visible effects of N/10 pottasium chloride is seen. These evidences will assume that there predominantly occurs in the ERG of *Eriocheir* process the corresponding PIII of vertebrate retina. When the eye was treated by one drop of ethylalcohol, irregular spike-like oscillations are superimposed on the retinal action potential at the falling phase of initial negative deflection and after the light is cut off. These oscillations may be regarded as the result of ganglionic activity by the administration of drug.

Summary

Action potentials in response to light flashes of long or short duration have been recorded on the isolated compound eye of *Eriocheir*. The ERG of the dark-adapted eye begins with a monophasic on-effect, the front of the visual elements being negative relatively to the back of the eye, rapidly returns to the base line during illumination, and ends in a complex off-effect. The light-adapted eye shows only a feeble on-effect. The course of dark adaptation was investigated. From the properties of the retinal response the author assumes that the ERG of *Eriocheir* consists of more than two components. These two components may be monophasic processes of the same electrical sign as demon-

strated by BERNHARD. The experiments suggest that there predominantly occurs a process which might be identical with PIII.

The author's many and hearty thanks are due to Prof. N. Y. KAWAMOTO, D. Sc. of our laboratory, and Dr. T. HANAOKA, Prof. of Nara Women's Univ., for their helpful criticism and advices, and especially to Mr. Y. MAEGAWA and Mr. K. YAMAZAKI for their much useful discussion.

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Pearl Industry and Research in Japan

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Pearls have been naturally produced in pearl oysters in various parts of the world. Only an extremely small proportion of the oyster population actually produces pearls. Artificial culture of pearls has therefore been attempted for a long time. According to the old records of Japan, the coastal zones of Nagasaki and Mie prefectures (especially the Shima. districts) had produced natural pearls though in very small numbers. This may be due to the fact that Japanese pearl oysters are found in the coast of Shima-district in greater numbers than any other part of Japan. Many scientists had been engaged in the study of artificial culture since the early years of the Meiji Era. In 1890, a theory on pearl formation was propounded by Mr. Masakatsu FUJITA, and in 1896, Mr. Kokichi MIKIMOTO succeeded in making the culture of semi-round pearls an enterprize which acquired the first patent for the process. In 1907 Dr. Tokichi, NISHIKAWA attempted to make round pearls artificially; since then various specialists have obtained as many as forty-nine patents, and now the industry is advancing as a unique one for Japan. The reason why the industry has been developed only in the country may be accounted for by two facts. The distribution of "Japanese pearl oysters" from which pearls are produced, are confined to Japanese Islands, and the whole process particularly, the insertion of the nucleus is quite delicate, and this is in accord with the nature of the Japanese. The economic development of the industry is mostly due to the high appreciation of pearls by foreigners, a large number being exported at a high price: In 1954 alone, $7,240,158 worth of pearls were exported to 52 countries including the United States. Pearls have thus occupied the highest position among all the marine products exported out of Japan.

In prewar days Europe and India were the best markets for Japanese pearls. While 19.6% of the Japanese cultured pearls in 1938 were bought by India, her purchase of this item dropped to 2.4% in 1950. But in the post-war world the United States of America has become the leading market for Japan's cultured pearls. In 1938 the United States of America took 39.1% of the cultured pearls exported out of Japan and in 1954, the percentage rose to 55.4%.

Problems in pearl culture:

As stated above, pearl culture is the favourite item in the export industry

of Japan. But to stabilize, develop and strengthen the industry, there are several problems to be solved. These problems are closely related to economic and scientific aspects. Special measures must be taken to maintain and raise the value of pearl as a jewel, rare as it is. At the same time, the culture process is based on biology, physics and chemistry, and there are many scientific problems to be studied. The following are the main problems:

a) Problems of the industry:

Pearls are appreciated by Westerners as a jewel next to the diamond, being used for necklaces and earrings, and are indispensable accessories of Western ladies. Besides, Westerners purchase jewels not only as accessories but also as treasures. It may be true, therefore, that if bad pearls be exported to foreign markets at random, persons who have possessed and loved good ones as well as jeweldealers and brokers who have bought them at comparatively high prices will be affected adversely and the trade will suffer. Thus the credit of the pearl as a jewel is likely to be lost in the long run. Regulatory measures detailed below are insisted on by many people to be taken at this juncture.

The activities of the pearl businesses may be grouped under the following categories:

1. Mother of pearl production
2. Pearl culture
3. Pearl processing
4. Export and selling
5. Nucleus manufactured and related business

Items 1 and 5 are often exercised by fisheries cooperatives other than the culturists, who purchase mother pearls collected by cooperatives in many cases. Small-scale businessmen are engaged in item 2 only, and some of them sell their products to processing factories without finishing. Protective measures against these activities are many and varied and the important ones are summarized below:

i. Finance:

It has been a feature in pearl culture that it could be undertaken on a comparatively small scale with a moderate capital. Of late, however, the price of mother pearls (nuclei) has risen exorbitantly; moreover, the saleable, finished products can be obtained only after three or four years from the time mother pearls are purchased. It is natural that financial stringency of the businessmen in the interval is very severe; to make matters worse, as pearls are not treated as mortgages in financial circles, they are forced to sell their incomplete products much against their will, and the lowering of quality is therefore likely. This situation is disastrous to the industry. To finance businessmen appropriately is an essential measure for maintaining the quality of pearls.

ii. Enforcement of license system:

Pearl raisers are growing in number year after year since the termination of the War, and there are now approximately 1,400 persons. Thus in spite of the decrease of the output to one-fifth of that of the prewar days, culturists have surpassed those in the past in their number. Because inexperienced, speculative persons have taken up the work charmed by the bright prospects for pearls, the quality of the latter is feared to go down. Licences must hence be given to those who have considerable experience, technical know-how and capacity.

iii. Planned production.

iv. Proper inspection system.

The products should be classified systematically and prices stabilized according to quality. All products should duly be inspected and those below the set standards be not allowed to be exported. This should be rigidly enforced and licences of those failing to observe these regulations should be cancelled.

To put these measures into practise, legal provisions are of course necessary, and special laws should be established.

b) Problems for Scientific Research:

As mentioned before, the pearl culture industry is largely dependent on the biology of the pearl oyster. Since the restoration of the Meiji Era, research has continuously been conducted on the biology and ecology of the pearl oysters by several scholars and enterprisers. But the efforts and results have been erratic and fragmentary. At this juncture the results obtained so far should be collated and reviewed by the competent authorities on this line. On the basis of the progress already made in such scientific studies, further research be carried out. The National Pearl Research Laboratory established in the spring of 1955 at Kashikojima, Mie Prefecture and other research laboratories attached to University of Fisheries should be able te attend to this.

The Problems for immediate consideration would be:

1. Artificial propagation of the mother oysters.
2. Improvement of pearl quality.
3. Adjustment of culture density.
4. Quality-improvement, copulation.
5. Improvement of fishing ground.
6. Forecast of sea-conditions.
7. Establishment of inspection system.

1. Artificial propagation of mother-pearls:

About 300,000 "kan" of mother pearl oysters used to be handled in pre-war days, but now the quantity has been increased to about twice of the amount. The supply of the mother pearls used to be dependent on the natural propagation Nowadays more than a dozen private laboratories including the Prefectural

Fisheries Experiment Station conduct researches on artificial propagation of the oysters with feeding of the larvae. In the studies on the larvae, however, careful surveys are needed, and the determination of the correct "propagation" time based upon the surveys is a fundamental item for attention. In view of the complex factors of oceanography, cooperative research on the biological and oceanographical aspects is necessary. At the same time, the cooperative research should not be fragmentary, as it is done now, but should be conducted on a wider and more coordinated basis.

2. Improvement of pearl quality:

With the discovery in 1907 of the cause of pearl formation, pearl culture was developed progressively by several students and businessmen including Mr. Kokichi MIKIMOTO. The theory of pearl formrtion, however, is a matter of controversy. Pearl enterprisers start the culture under apparently similar conditions. A variety of pearls—some costing tens of thousands of yen, and others of little value—may be produced. Thus pearl culture still remains a speculative enterprise. The factors affecting the color and brightness, which appear to be caused by secretions of the oyster are little understood. Researches are being carried out by the National Pearl Research Laboratory, Mie Prefectural Fisheries Experiment Station, Nippon Institute for Scientific Research on Pearls and Mie Prefectural University.

i. *Histochemical Research on pearl layer formation.*—The process of shell and nacre formation is considered to be a metabolic function of the epithelia of the mantle and of the pearl sac. As for the mechanism of the deposition of the calcium carbonate into the shell, the catalytic action of carbonic anhydrase may be related to that mechanism. The activities of carbonic anhydrase are very striking in the outer epithelium of the mantle and in the pearl sac, and less in the connective tissues and the mucous cells. By the microinceneration method, the calcium contents of the mantle eipthelium and pearl sac are determined and they are prominently distinguishable in the outer epithelia of the mantle and of the pearl sac. The formation of protein, which is one of the components of nacre of the shell is still in question. However, Ribo nucleic acid is distinguished in the mantle by pyronin-methylgreen staining method, and it would synthesize the protein in the cytoplasm of those tissues.

These facts indicate that calcium and carbonic anhydrase as well as Ribo nucleic acid exist abundantly in the outer epithelia of the mantle and pearl sac. But the manner of action of these substances in the formation of the nacre of the shell and of the cultured pearl itself still remains to be studied.

ii. *Research on Pearl formation with the application of "Isotopes":*— The chief chemical composition of pearl is calcium. It is most important to develop the cultivation technique, ascertaining what tissue of the pearl oyster

shell (*Pinctada martensii* D.) mostly accumulate the valuable calcium absorbed in the flesh, and how the calcium deposit forms a pearl. Moreover, independent surveys of the distribution of calcium in the sea-water environment in the areas concerned might be helpful in fixing suitable grounds for the operations. These researches are beset with difficulties, but by the use of Radio Isotopes we can study the movements of calcium by observing its radioactivity. In 1949 the Mie Prefectural Fisheries Experiment Station received the calcium Isotope from SCAP for the study. The station has achieved some extremely interesting results, and assidous research has been carried out in the Faculty of Fisheries, Prefectural University of Mie.

iii. *Spectroscopical Research on metal "Ion" content.*—The colour and gloss of pearl appear to be governed by the metal Ion contained in it. Spectroscopical research on this has recently been carried out by the Kyoto University, and the National Pearl Research Laboratory. The progress of this type of research and studies on the distribution of Ion in the sea-water are expected to assist the scientific culture of pearls.

iv. *Crystolographical studies of pearl.*—From crystolographical observations of the surface structure of the nacreous layer, we can distinguish the unconcentric accumulation of scaly laminae measuring 0.5μ in thickness, composed of conchiolin membrane and argonite crystals deposited on the surface. The lamellar lines observed in sections of the pearls are not continuous, and, the margins of these laminae are usually exposed to the surface of the pearls exhibiting volute or parallel patterns. Although the colour of the pearls may depend on the chemical composition and the mode of accumulation of the laminae, the lustre is chiefly influenced by the surface constitution.

Further, the three developmental stages in the process of crystallization of the argonite are recognizable with the help of the electron-microscope. The final developmental stage of crystal growth shows the regular, hexagonal, platy, idiomorphic aragonite crystals on the entire surface of the pearl, producing the shining lustre. These problems are carried out in the Prefectural University of Mie.

3. Adjustment of cultivation density:

The pearl oyster subsists on plankton in the sea-water and there would hence appear to be a limit to the density of mother-oysters to be cultivated in a given area of water. The factors of potentiality of cultivation are, however, not fully known and cultivators follow their own judgement. Therefore, the density of cultivation is often not adjusted properly and consequently, the quality of the pearls has been adversely affected. Oceanographic surveys required for determining questions of cultivation densities would involve considerable expenditure which the private businessmen can hardly afford. This work has thus

received least attention. Regular observations of the fishing ground are only conducted by the Prefectural Fisheries Experimental Station. Further systematic research in oceanography and biology must, therefore, be established.

4. Quality Improvement with cross-bred oysters:

Even the biggest pearl obtained from the pearl oyster measures only about 9 mm in diameter, the dimensions of the pearl being limited by the size of the oyster. Since the value of a pearl depends to a great extent on its size, attempts are being made to obtain bigger pearls by providing bigger oysters through cross-breeding between "*Pinctada martensii*" and "*Pinctada margritifera*". The work is still in its beginning; but considering the far reaching achievements of cross breeding in agriculture, the subject of cross breeding oysters should receive considerable attention.

5. Improvement of fishing-grounds:

In the Ago bay, which is the principle ground for pearl production, the water temperature lowers in winter, and there is a danger of the adults and young of oysters being killed by the socalled "cold tide". To prevent this the Fukaya channel has been excavated to lead the warmer sea-water into the bay, but so far no objective has been attained. It is necessary to develop the channel for this purpose. On the other hand, however, an excessive inflow of sea-water might adversely affect the pearl quality. The optimum conditions to be ensured in the channel have hence to be ascertained. The agencies concerned are now studying the problem from the view-point of oceanography and biology including the nature and behaviour of the marine plankton, the bottom-living animals and sea-weeds.

This work will greatly influence the production of the Ago cove in the future and an active programme of research should necessarily be pursued.

6. Forecast of sea conditions:

The highly variable conditions of the sea, the occurrence of brown waters and cold tides, the rise and wane of the black current—all these have far-reaching effects on pearl culture. Timely prediction of such changes and other phenomena would greatly assist the pearl cultures. Proper oceanographic research including a special study of the black current is therefore an essential step towards the development of the pearl industry in Japan.

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アミラン網糸の加熱による影響について

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Effect of Heating on the Physical Properties of Amilan cord

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Résumé

We had the experiments on the physical properties of 250 *D* 2/6 Amilan netting cord (1st twist: left handed 296±4.3, 2nd twist: right handed 168±2.3 for 50 cm. long.) disposed by heating at various temperatures and for varied times.

After the disposition with a drier at each temperature and respective time, the tensile strength, the elongation, the length, the pitch, the diameters of cord and of filament were measured, and the colours of the cord were surveyed.

From these experiments, the following results were obtained.

1) At higher than 120°C, the tensile strength and the elongation decrease with the increasing of temperature and of time, and the variant coefficients of the tensile strength and of the elongation increase in accordance with the temperature and the time.

2) The length and the pitch are gradually contracted almost in proportion to the temperature rising; those ratios are in a near resemblance to each other.

3) The diameter of the cord has not much of a change, but the size of the filament fattens with the temperature increases.

合成繊維漁網の編網後の目締めのために網地に加熱したり，又溶融紡糸後の熱延伸を撚糸にも拡張して，撚糸の強力増加とヤング率の変化から結節部のずれ即ち“目ずれ”を防ぐ方法が各種の漁網用合成繊維について最近行われている。これらの操作は所謂熱固定操作であり，撚糸の種類に応じて加熱温度と加熱時間とを適当に定める必要がある。著者の一人石井[1]はさきに加熱温度による物理的変化について述べたが，今回は主として各温度による処理時間の影響について実験を行つたので，その結果について報告する。

実験：供試材料はアミラン 250 *D* 2/6 網糸（50 cm 間の下撚：右 296±4.3，上撚：左 168±2.3）を用い，加熱に際しては温度と時間とを種々変化して処理した。即ち直径ピッチ及び長さを測定した試料を所要温度に調節したアクメ乾燥器内に入れ所定時間毎に取出し，2 時間空中に放置した後再び直径・ピッチ及び長さを測定し，然る後抗張力並びに伸度の測定を行つた。尚処理網糸の一部試料をほごしてフイラメントを取出し，顕微鏡下（×800）でその直径を測定した。実験中の室内平均温度は 16.4°C，関係湿度は 63～73% であつた。

第 1 表は各処理に於ける抗張力．切断伸長及び長さの変化で，第 2 表は撚糸の直径とピッチの変化を示し，第 3 表はフイラメントの直径の測定値で，夫々 20 本宛の平均値である。

Table 1. Changes of the tensile strength of the elongation at break and of the length.

Standard			
	T_0 kg 4.80	E_0 cm 5.43	l_0 cm 30.00
	σ .183 (N=100)	σ .485 (N=100)	σ .044 (N=600)
	V ‰ 38	V 89	V 1.5

Temp.	min.	10	20	30	40	50	60
80°C	T	4.68 kg	4.70	4.76	4.70	4.60	4.61
	σ	.205	.194	.134	.165	.251	.188
	V	44 ‰	41	28	35	55	41
	T/T_0	98 %	98	99	98	96	96
	E	5.29 cm	5.13	5.29	5.47	5.30	5.10
	σ	.419	.341	.338	.482	.504	.383
	V	79 ‰	66	64	88	95	75
	E/E_0	97 %	94	97	101	98	94
	l	29.62 cm	29.69	29.66	29.74	29.86	29.82
	σ	.064	.064	.049	.077	.051	.068
	V	2.2 ‰	2.2	1.6	2.6	1.7	2.3
	l/l_0	99 %	99	99	99	100	99
100	T	4.82	4.68	4.94	4.77	4.59	4.69
	σ	.134	.168	.205	.272	.235	.151
	V	28	36	42	57	51	32
	T/T_0	100	98	103	99	96	98
	E	5.74	5.35	5.81	5.62	5.42	5.42
	σ	.364	.387	.374	.556	.437	.463
	V	63	72	64	99	81	85
	E/E_0	106	99	107	103	100	100
	l	29.40	29.35	29.48	29.52	29.16	29.34
	σ	.053	.101	.063	.053	.070	.075
	V	1.8	3.4	2.1	1.8	2.4	2.6
	l/l_0	98	98	98	98	97	98
120	T	4.74	4.73	4.72	4.66	4.63	4.28
	σ	.151	.142	.193	.243	.216	.345
	V	32	30	41	52	47	81
	T/T_0	99	99	98	97	96	89
	E	5.68	5.64	5.55	5.48	5.44	4.63
	σ	.356	.253	.424	.475	.335	.514
	V	63	45	76	87	62	111
	E/E_0	105	104	102	101	100	85
	l	29.06	29.21	29.12	29.04	28.81	28.90
	σ	.103	.082	.077	.062	.075	.061
	V	3.5	2.8	2.6	2.1	2.6	2.1
	l/l_0	97	97	97	97	96	96
140	T	4.58	4.38	4.37	3.73	3.00	2.73
	σ	.174	.208	.260	.288	.252	.261
	V	38	47	59	77	84	96
	T/T_0	95	91	91	78	62	57
	E	5.82	4.88	5.28	4.22	3.52	3.14
	σ	.480	.352	.551	.423	.325	.294
	V	82	72	104	100	92	94
	E/E_0	107	90	97	78	65	58
	l	28.52	28.56	28.65	28.68	28.29	28.33
	σ	.084	.120	.084	.089	.078	.058
	V	2.9	4.2	2.9	3.1	2.8	2.0
	l/l_0	95	95	96	96	94	94
160	T	3.77	3.65	3.40	2.67	1.74	1.95
	σ	.348	.326	.258	.268	.280	.419
	V	92	89	76	100	161	215
	T/T_0	79	76	71	56	36	41
	E	4.53	4.37	3.88	3.12	2.58	2.61
	σ	.263	.513	.230	.269	.293	.546
	V	58	117	59	86	114	209
	E/E_0	83	80	71	57	48	48
	l	27.90	27.66	28.14	28.08	27.26	27.87
	σ	.012	.095	.058	.035	.149	.101
	V	0.4	3.4	2.1	1.2	5.5	3.6
	l/l_0	93	92	94	94	91	93

T; Tensil strength E; Elongation at break in cm. to initial 20 cm long σ; standard devation
V; Variant coefficient N; Number of observation T_0, E_0 and l_0 were measured in temp. 115.0°C

Table 2. Changes of the diameter and of the pitch of 250 *D* 2/6 Amilancord

Standard		$D_0=0.740^{mm}$			$p_0=2.99^{mm}$		
Temp. \ min.		10	20	30	40	50	60
80°C	D	0.742	0.744	0.744	0.725	0.748	0.739
	D/D_0 %	100	101	101	98	101	100
	p	2.96	2.99	2.98	2.94	2.95	2.93
	p/p_0 %	99	100	100	98	99	98
100	D	0.736	0.761	0.738	0.732	0.732	0.734
	D/D_0 %	99	103	100	99	99	99
	p	2.89	2.92	2.97	2.96	2.87	2.98
	p/p_0 %	97	98	99	99	96	100
120	D	0.737	0.745	0.742	0.742	0.737	0.757
	D/D_0 %	100	101	100	100	100	102
	p	2.91	2.86	2.93	2.89	2.89	2.88
	p/p_0 %	97	96	98	97	97	96
140	D	0.738	0.746	0.742	0.743	0.732	0.736
	D/D_0 %	100	101	100	101	99	99
	p	2.90	2.92	2.91	2.86	2.79	2.80
	p/p_0 %	97	98	97	96	93	94
160	D	0.733	0.746	0.734	0.750	0.742	0.736
	D/D_0 %	99	101	99	101	100	99
	p	2.84	2.84	2.80	2.82	2.76	2.78
	p/p_0 %	95	95	94	94	92	93

D; Diameter of cord, *p*; pitch

Table 3. Changes of the diameter of filament drew out of 250 *D* Amilan yarns

Standard		$f_0=0.0429^{mm}$ $\sigma=.00311^{mm}$ ($N=50$)					
Temp. \ min.		10	20	30	40	50	60
80°C	f	.0425mm	.0442	.0422	.0432	.0448	.0424
	σ	.00405	.00357	.00279	.00315	.00411	.00326
	f/f_0	99 %	103	98	101	104	99
100	f	.0442	.0438	.0444	.0441	.0426	.0424
	σ	.00318	.00361	.00374	.00356	.00357	.00225
	f/f_0	103	102	103	103	99	99
120	f	.0438	.0446	.0436	.0433	.0462	.0458
	σ	.00435	.00244	.00306	.00331	.00484	.00423
	f/f_0	102	104	102	101	108	107
140	f	.0436	.0429	.0434	.0427	.0434	.0436
	σ	.00300	.00502	.00314	.00281	.00347	.00204
	f/f_0	102	100	101	100	101	102
160	f	.0449	.0462	.0462	.0447	.0475	.0459
	σ	.00288	.00316	.00468	.00416	.00345	.00321
	f/f_0	105	108	108	104	111	107

f; diameter of filament　　σ; standard deviation

抗張力及び伸度の変化: 第 1 図及び第 2 図は加熱温度と加熱時間別の抗張力並びに伸度の変化で，この図からわかるように抗張力伸度は共に 100°C までは殆んど変化しないが，120°C を超えると大体処理時間の増加に比例して減少する傾向が見られる。しか

もこれら減少の傾向はさきに得られた実験の結果[1]と大体同じようである。尚第 1 表より知られる通り加熱温度が大体 120°C 以上になると，抗張力の変異係数は加熱温度並びに加熱時間の増加するに従つて段々大きくなる。即ちアミラン網糸の抗張力は段々不揃さを増してくるようである。

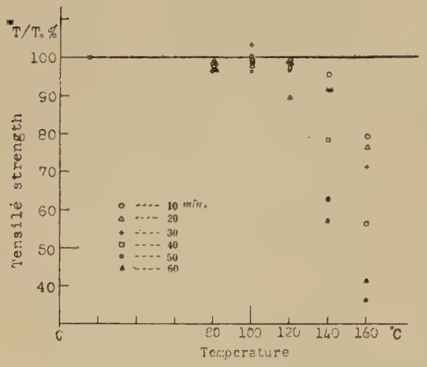

Fig. 1 Relation between the tensile strength in each temperature and the time of heating.

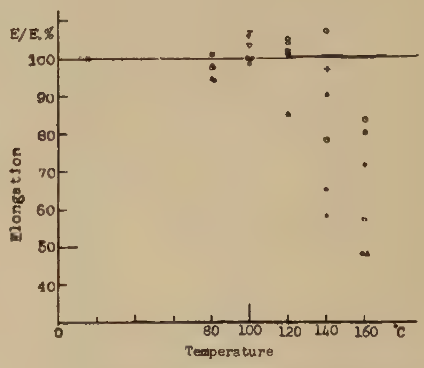

Fig. 2. Relation between the elongation in each temperature and the time of heating.

ピッチ及び長さの変化: 第 3 図に示すように，ピッチ及び長さの変化はこの実験の範囲内では温度と共に略直線的に収縮し，しかもピッチの収縮率と長さの収縮率とは殆んど変りないことがわかる。又長さの変動は加熱時間による差は殆んど見られないが，加熱温度が高くなる程収縮率が大きいことが認められる。

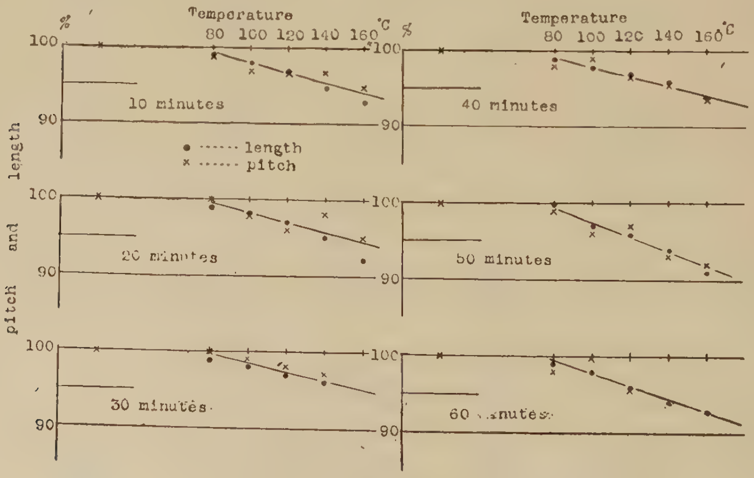

Fig. 3. The change of the length and it of the pitch.

直径の変化: 第 4 図は網糸の直径の変化とフイラメントの直径の変化を示す。この実験の範囲内では，網糸の直径は加熱によつて殆んど変化が認められないが，フイラメントの直径は温度と共に増加する傾向が見られる。このことは一見不合理のように考えられるが，おそらく網糸の機構に重要な関係があるのではないかと思われる。この問題については

The change of color of Amilan 250D 2/6 netting cord by heating

今後検討して見る考えである。

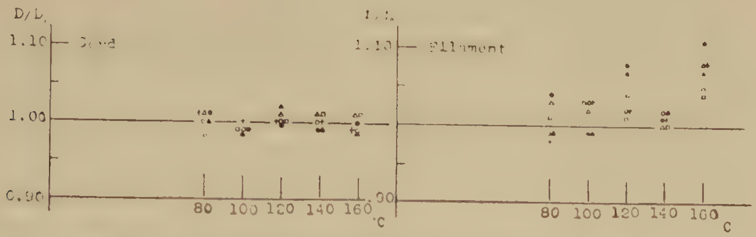

Fig. 4. Changes of the diameter of cord and of filament. Every marks were written in proportion to Fig. 1.

其の他：加熱による糸表面の変色の模様は図版に示す通りで，温度 100°C までは殆んど変らないが，120°C のときは時間 20 分頃から僅かに薄黄色を呈し初め，140°C の場合は加熱時間を増すに従つて漸次淡黄色から薄茶色にかわり，160°C においては薄茶色—茶色—茶褐色と漸次変化することが認められる。

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養鰻池の水変りに関する研究

II. Brachionus plicatilis (輪虫類) によるプランクトン植物の捕食作用 (水変りの 1 因として)

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Studies on the "Mizukawari" in Eel-culture Ponds

I. The Feeding Activity of Brachionus plicatilis on Phytonannoplankton (As a cause of "Mizukawari")

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Résumé

On the west coast of Ise Bay, in Mie Prefecture, there are about 200 of eel-culture ponds; these ponds are found near the coast line, with considerable salts (Cl 0.2～8‰) in their water, belonging to the category of brackish-water.

In these eel-culture ponds we have seen the "summer kill", what we call the "Mizukawari", phenomena, which mean the sudden decrement of phytoplankton, the change of the water colour of green into transparent with the sudden rise in its transparency, and that the conspicuous decrease in pH and the dissolved oxygen, consequently to cause the death of the eels as well as carps and mullets cultured in the ponds. In most cases of the "Mizukawari" phenomenon, it comes to happen with an abnormal reproduction of *Brachious calyciflorus* or *B. plicatilis* (both, rotifer), which INABA (1937) took as a result of "Mizukawari", not of its cause.

Here, the author will report the successful results of his experiments, in which he could materialize in his laboratory the conditions of "Mizukawari" by the abnormal reproduction of *Brachionus plicatilis* and its conspicuous feeding on phytonannoplankton.

The results are:

1. When *Brachionus plicatilis* in various population densities is added into the water of eel-culture pond with conspicuously increased *Synechococcus* sp. (Cyanophyceae) of phytonannoplankton, *Synechococcus* decreases in number in proportion to the density, the water colour changes from green to yellow brown and eventually into transparent; *Brachionus plicatilis* itself show its enormous reproduction, the digestive system of which changes into green by devouring the micron-algae there.

2. When the water containing *Brachionus plicatilis* of great increased number is filtered or boiled, is poured adding into the water with the micron-algae, there can be seen no decrease of *Synechococcus* sp.

3. This will consequently prove that this phenomenon of "Mizukawari" which results in the conspicuous decrease of *Synechococcus* sp. (micron-algae) should be caused directly by the abnormal reproduction and its feeding activity.

養鰻池の"水変り"には多くの場合，輪虫類の *Brachionus calyciflorus*[1], *B. urceolaris* の大増殖の伴うことは稲葉 (1934) が最初に静岡県吉田地方から報告し，この特異性を稲葉峻 (1937) は静岡県浜名地方で確認し，この輪虫類の異常増殖は植物性プランクトンの消滅による"水変り"の結果であると考えた。伊藤 (1955) は三重県津附近の養鰻池におけるプランクトン動物の分布と池水の塩分との関係を明かにし，更に水変り時には，低鹹の池では *B. calyciflorus*, 稍々高鹹な池では *B. plicatilis* の大増殖の見られることを報告した。

其の後筆者は養鰻池の植物性プランクトンの培養実験中，*Brachionus plicatilis* の発生した培養液では間もなく植物性プランクトンが消滅し水が澄んで来ることを発見し，更に植物性プランクトンが増殖し緑色を呈する池水 100 cc に *B. plicatilis* を約 1000 個体入れたところ 2 日後には完全に緑色はなくなり池水は透明になるのを見た。この輪虫類を検鏡したところ消化管は著しく緑色に着色していた。恐らくこの輪虫類の異常増殖とその植物性 nannoplankton の捕食作用が養鰻池における"水変り"の 1 因をなすのではないかと思われたので，この実験を行つた。

材料並に実験方法

プランクトン植物の材料は津市島崎町黒宮辰次郎氏所有の養鰻池 (第 5 号池: 池水の塩素含有量 Cl=3.616‰) のものを用いた。この池の植物性プランクトンは藍藻類の *Synechococcus* sp.[2] のみから成り，著しく繁殖し鮮緑色を呈し (平均細胞数 7.67×10^6 /cc), 動物性プランクトンは殆んど認められなかつた。池水を実験室に持参し，プランクトン・ネットで動物その他の大型 seston を取除き，その濾液に KNO_3 並に KH_2PO_4 溶液を夫々 18.7 mg/L (N として), 1.3 mg/L (P_2O_5 として) 程度に加え 1 日放置して実験に供した。

輪虫類，*Brachionus plicatilis* の材料は津市栗真町屋町加藤慎太郎氏所有の養鰻池 (第 2 号池: 池水の塩素含有量 5.512‰) から実験当日に採集したのを用いた。この池は水変り中であり，プランクトンは殆んど全部輪虫類から成り，その中 *B. plicatilis* が約 90% を占めていた。プランクトン・ネットで集め池水に濃縮して実験室に持参し実験に用いた。

前記の植物性 nannoplankton, *Synechococcus* sp. が著しく増殖し，鮮緑色を呈する池水を，200 cc 容量の三角フラスコに 100 cc とり，その各々に *B. plicatilis* を 10, 100, 500, 1000, 3200 個体の割合に夫々水変り池水と共に加えた。即ち予め *B. plicatilis* の平均個体数密度を算定しておき，所要の推定個体数に相当する水量を注加した。更に *B. plicatilis* が何かの毒素を分泌し，或いは水変り池水そのものが *Synechococcus* sp. に影響を与えるのではないかとの疑問もあつたので，*B. plicatilis* の濾液並にその煮沸液を夫々 0.1, 1.0, 10.0 cc を加えてその影響を見た。濾液は前記の輪虫類を含んだ水変り池水を東洋濾紙 No. 5 で濾したものであり，煮沸液はそれを 5 分間煮沸して後放冷しその上澄液を用いた。

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1) 従来は *Brachionus pala* として用いられているが，最近 (山元，1949) では，*B. calyciflorus* が正しい。

2) 種名の同定は横浜市立大学文理学部生物学教室福島博氏を煩わした。ここに感謝の意を表します。

プランクトン植物，*Synechococcus* sp. の細胞数の計数には Thoma-Zeiss の血球計算盤を用いた。即ち試水 2cc を駒込ピペットでとり，10cc 容量の沈澱管に移し，フォルマリン 3 滴を加えて遠心分離器（電動式，2000回/分，15 分間）で沈澱させ，それを 1cc に濃縮し，細胞を管中に一様に分布させ，その 1 部をとり，血球計算盤に移し，両対角線上の小区劃 32 個の細胞数を計数した。細胞数の少い場合には試水 4cc をとり，1cc 或いは 0.5cc に濃縮し，計算盤の大区劃を単位として 16 区劃の細胞数を計数した。いずれの場合も池水 1cc 中の細胞数として算出した。

実験は 6 日後に終了し，三角フラスコにフォルマリンを加えて *B. plicatilis* を固定し，遠心分離器にかけて全部を沈澱させ，その 1/5 をとつて計数盤に入れて個体数を計数し，*B. plicatilis* の 6 日間の全個体数を斃死と生存に分けて算出した。

実験は昭和 30 年 6 月 21 日から 27 日に亘つて行い，水温は 26.1〜28.1°C であつた。

実験結果

本実験の結果は Table 1, Fig. 1 に示した。

Table 1. The reductions of *Synechococcus* sp. (phytoplankton, blue-green algae) by the feeding of *Brachionus plicatilis* (Rotifer).

	Bottle Number	Treatment	Number of cells, *Synechococcus* sp. /cc					Number of *B. plicatilis* after 6 days, per 100cc.		
			Before	After						
				2 days	3 days	4 days	6 days	Alive	Dead	Total
Living specimen of *B. plicatilis*		Added No.	×10⁶ → $\times10^6$	$\times10^6$	$\times10^6$	$\times10^6$	$\times10^6$			
	1	10	7.56	11.00	—	12.19	1.12	13035	0	13035
	2	100	8.13	10.69	10.44	6.06	0.84	11910	0	11910
	3	500	7.06	8.56	7.69	0.63	0.25	9024	576	9600
	4	1000	8.50	7.56	2.50	0.31	0.21	6872	1938	8810
	5	3200	7.63	2.31	0.44	0.22	0.11	3398	4152	7550
	6	0 (control)	7.13	10.44	—	14.69	12.50	0	0	0
Filtered water		Added vol.	$\times10^6$	$\times10^6$		$\times10^6$	$\times10^6$			
	7	0.1 cc	6.44	10.69	—	13.44	5.06			
	8	1.0	7.94	8.69	—	12.63	8.44			
	9	10.0	7.75	7.94	—	9.13	5.91			
Boiled water		Added vol.	$\times10^6$	$\times10^6$		$\times10^6$	$\times10^6$			
	10	0.1 cc	6.88	8.19	—	11.31	9.69			
	11	1.0	8.75	7.81	—	10.50	11.80			
	12	10.0	7.69	8.19	—	11.98	4.94			

1. 水色の変化

No. 5（*B. plicatilis* 3200 個体/100cc，以下 R. 3200 と略記する）のみは 1 日後から緑色を減じ，黄緑色となり著しく澄みはじめ，2 日後にはこの輪虫の運動状況を透視出来る位に透明になり，それから 6 日後まで透明度を増し，水色は無色に近くなつた。No. 4（R. 1000）は 2 日後に澄みはじめ 3 日後には著しく透明になつた。No. 3（R. 500）は

3 日後から稍々澄みはじめ，4 日後には殆んど透明になつた。No. 2 (R. 100) は 4 日目の朝には稍々透明になりつつあつたが，その日の午後（約 7 時間後）には著しく透明になり，No. 1 (R. 10) は 4～5 日後から澄みはじめ 6 日後には急激に透明度を増した。このように水色の緑色から無色への変化並に透明度の変化は，最初に加えた *Brachionus plicatilis* の個体数に比例している。

これに対し control (No. 6) は実験前に加えた KNO_3, KH_2PO_4 の効果のためか却つて緑色を増した。一方 *B. plicatilis* の濾液並に煮沸液を加えたものは，No. 9 並に No. 12 のみは 6 日後に稍々緑色を減じ澄んで来たが，他はいずれも control と大差はない。

全体的に見ると，*B. plicatilis* を加えることによつておこる水色の変化系列は，鮮緑色から稍々褐色を帯びた黄緑色となり，次第に水は透明になり，6 日後には殆んど無色透明になる。

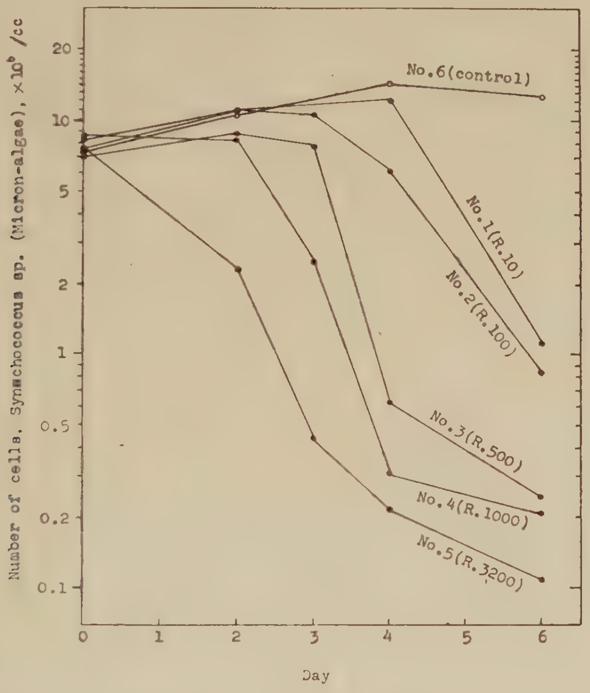

Fig. 1. The decreases of *Synechococcus* sp. (Phytoplankton) by the feeding of *Brachionus plicatilis* (Rotifer)

2. *Synechococcus* sp. の細胞数の変化

Table 1 並に Fig. 1 に明かのように，先ず control (No. 6) においては細胞数は 4 日後まで増加し，6 日後は稍々減少したが実験前より却つて増加している。これは加えた KNO_3, KH_2PO_4 の効果によるものであろう。これに対し，*B. plicatilis* を加えたものは，水色の変化と同様に，最初に加えた輪虫の個体数密度に比例して *Synechococcus* sp. の減少率は大きい。その減少状況を稍々詳細に見ると，No. 5 (R. 3200) では細胞数は 2 日後には実験前の約 1/3 に減少し，3 日後には 1/17 に，4 日後には 1/35 に，6 日後には 1/70 に減少し，最初 7.63×10^6 細胞が 6 日後には 0.11×10^6 細胞/cc になつた。

No. 4 (R. 1000) は 2 日後までは殆んど減少は見られないが，3 日後には急激に減少し，4 日，6 日後には夫々最初の 1/27，1/40 に減少した。No. 3 (R. 500) は 3 日後までは殆んど変化は見られないが，4 日後から急激に減少し，6 日後には 1/30 に減じた。No. 2 (R. 100) は 3 日後までは実験前より細胞数は却つて増加しているが，4 日後から減少し，6 日後には最初の約 1/10 に減少した。No. 1 (R. 10) は No. 2 と同様に 4 日後までは却つて増加しているが，6 日後には急激に減少し最初の細胞数の 1/7 に減少した。

次に *B. plicatilis* の濾液並に煮沸液を加えたものはいずれも 4 日後までは実験前より著しく増殖を示した。しかし全体的には 6 日後には稍々減少し，特に濾液並に煮沸液の夫々 10 cc を加えた No. 9, No. 12 の減少が稍々顕著であつたが，*B. plicatilis* を加えたものに比べるとその減少率は極めて小さい。

3. *Brachionus plicatilis* の個体数の変化

10, 100, 500, 1000, 3200 個体/100 cc の 5 種の個体数密度で加えた *B. plicatilis* の増殖速度はこの実験では明かにすることは出来なかつた。しかし 6 日後の *B. plicatilis* の全個体数は Table 1 の最後の欄に生存，斃死個体に分けて掲げた。この中には *Synechococcus* の計数の際の 4～5 回，6～10 cc の採水中の *B. plicatilis* の影響は考慮していないが，*B. plicatilis* の増殖力は驚くべきものであることが分る。

6 日後に *B. plicatilis* を固定し，消化管の内容物の着色状況を検鏡したところ，No. 5 の生残個体の消化管はすべて無色でプランクトン植物による緑色は見られず実験前の状態にかえつていた。No. 4 並に No. 3 の消化管の内容物は黄緑色が主で，1 部鮮緑色を混じていた。これに対し No. 2 並に No. 1 では消化管は著しい緑色を呈し，固定沈澱させたものもそのため緑色を呈していた。従つて上述の *B. plicatilis* の死亡は over population による食餌欠乏とその他の環境条件の変化に起因すると考えられる。

考　　察

この実験における養鰻池の植物性プランクトン，*Synechococcus* sp. の短時日の著しい減少の原因としては，第 1 に輪虫類の *B. plicatilis* が何らかの毒素を分泌するか，或いは代謝生成物質による影響，第 2 に *B. plicatilis* の大増殖による水質の変化の影響，第 3 に *B. plicatilis* の直接的の補食作用の 3 つが考えられる。しかし *B. plicatilis* の異常増殖した水変り池水の濾液並に煮沸液を加えても *Synechococcus* の著しい減少は見られないのみか，却つて全体的には増加する傾向がある。また *Synechococcus* の斃死による緑色沈澱は認められない（フォルマン並にアルコール等を加えると斃死し間もなく緑色沈澱を生ずる)。これ等の点から見て，第 1 の原因は否定される。一方実験に用いた *B. plicatilis* は水変り池のものであり，検鏡したところでは，消化管の内容物は無色で植物性プランクトンの着色は見られなかつたが，これを *Synechococcus* の著しく増殖した池水に加えると間もなくその消化管は緑色に着色し，その上 *Synechococcus* の多い間は消化管は緑色であるが，それが減少し，水が透明になるにつれて，胃内容物は緑色を失い，黄緑色，黄褐色に変じ，6 日後には全く無色透明になり，実験前の状態にかえる。また *Synechococcus* の減少率は，*B. plicatilis* の個体数密度に略々比例している (Fig. 1)。これ等の点から考えて，植物性プランクトンの *Synechococcus* の著しい減少は，第 2，第 3 の原因，特に *B. plicatilis* の直接的の捕食作用によると考えてよいであろう。

次に捕食者としての *B. plicatilis* と被捕食者としての *Synechococcus* sp. との体の大きさの関係を見ると，*B. plicatilis* にはいろいろの成長段階のものが混在し，大きさは均一ではないが，被甲長 124～285 μ，胃は略々球形を呈するが，稍々扁平で，長径 50～100 μ，短径 37～74 μ で，胃の容積は平均 $2\times10^5\ \mu^3$ である。*Synechococcus* sp. の細胞は円筒状を呈し，単体か或いは 2 個の連体を作る。所謂 Micron-algae に属し，ブラウン運動を呈するので精密な測定は困難であつたが，普通大きさは 1～2 μ で，1 細胞の容積は大きく見積つて 2 μ^3 程度である。

B. plicatilis 1 個体 1 日間の平均捕食量は，本研究では明かにすることは出来なかつたが，第 2 報の結果によつて概算すると 0～1 日後が最も大きく，20×10^4 細胞，1～2 日後は 8.5×10^4 細胞，2～3 日後は 0.1×10^4 細胞となり急激に減少している。従つてこの実験における *Synechococcus* sp. の短時間の急激な減少は，*B. plicatilis* の直接的捕食作用に依存すると見てもその可能性は考えられないことはない。

最後に本実験における *Brachionus plicatilis* の捕食作用によつて生ずる水色，透明度，植物性プランクトンの変化速度，並に *plicatilis* の個体数密度の状態は，天然の養鰻池における *B. plicatilis* の大増殖を伴つた“水変り”のそれに極めてよく類似している。恐らく *B. plicatilis* の大増殖とその植物性 nannoplankton の捕食作用が“水変り”と密接な関係にあるのではないかと思われる。pH 並に溶存酸素の変化に関しては第 2 報に掲げた。

摘　　要

1)　養鰻池の“水変り”には多くの場合，輪虫類の *Brachionus plicatilis* 或いは *B. calyciflorus* の異常増殖を伴う。

2)　Micron-algae に属する植物性プランクトン，*Synechococcus* sp. のみが著しく増殖し，鮮緑色を呈する池水に *B. plicatilis* をいろいろの個体数密度に加えると，その密度に比例して *Synechococcus* は急激に減少し，1～2 日の間に水色は緑色から黄褐緑色を経て無色透明となり，一方 *B. plicatilis* もまた急激に大増殖を行い，なおその消化管の内容物は緑色になる。

3)　*B. plicatilis* の異常増殖した水変り池水の濾液並に煮沸液を加えたもの，*B. plicatilis* を加えないものでは *Synechococcus* の減少は見られない。

4)　従つて *Synechococcus* sp. の著しい減少に伴う“水変り”類似の現象は，*B. plicatilis* の異常増殖とその顕著な直接的の捕食作用に原因すると考えられる。

最後に本研究に種々の助言を与えられた本学水産学部長岡田弥一郎教授に謝意を表します。

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養鰻池の水変りに関する研究

II. Brachionus plicatilis による水変りにおける pH・O_2 の変化

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Studies on the "Mizukawari" in Eel-culture Ponds

II. The Changes in pH and O_2 in the "Mizukawari" by Brachionus plicatilis

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Résumé

In the preceding paper (ITO, 1955b), the author reported his successful results from materializing the experimental "Mizukawari" phenomenon in the eel-culture ponds, of the abnormal reproduction of *Brachionus plicatilis* belonging to the brackish-water rotifer and its feeding activity on phytonannoplankton.

This is the report of the details from his researches in the changes in pH and the dissolved oxygen of the water and of the population density of the rotifer itself, which will be caused in accompany with the abnormal reproduction of *Brachionus plicatilis* and its feeding activity.

1. When *Brachionus plicatilis* is added at the rate of 30 individuals/cc into the green water of eel-culture pond with the conspicuously increased *Synechococcus* sp. belonging to Micron-algae (13.38×10^6 cells/cc), the *Synechococcus* shows a conspicuous decrease starting with the next day, and 3 days later it reduces to 0.066×10^6 cells/cc, about 1/200 of it before the experimental process, with the water colour changed into transparent.

2. While, *Brachionus plicatilis* itself will show a sudden increase up to its maximum of 102 inds./cc, by the 3rd day after the addition; but starting with the 4th day it begins to show a sudden decrease from its death and will be nearly destroyed to leave 0.7 inds./cc in 7 days.

3. In accompany with it, pH of the water decreases to 6.9–7.3, and the dissolved oxygen also conspicuously reduces down to 3–4 cc/L (50–70%).

4. Adopting 22 instances of the "Mizukawari" phenomena in the eel-culture ponds of Mie and Shizuoka prefectures, the peculiarities of their "Mizukawari" phenomena were induced from water colòur, transparency, plankton, pH and dissolved oxygen in the water, and compared with those resulted from the experiments: the results proved the similar "Mizukawari" phenomenon by the abnormal reproduction of *B. plicatilis* and its feeding activity on micron-algae in the laboratory to be well in accordance with that of the natural eel-culture ponds.

5. Consequently, it will be concluded that the abnormal reproduction of the rotifer in eel-culture ponds at the time of "Mizukawari" would not be the result of the "Mizukawari" from the disappearance of phytoplankton by the want of nutrients, but the very cause of it.

第1報（伊藤 1955b）に，*Brachionus plicatilis* の増殖力並に植物性ナンノプランクトン，*Synechococcus* sp. の捕食作用が極めて顕著で，1～2 日の間に水色は無色透明に近くなり，養鰻池における“水変り”に酷似する現象を実験室で作ることに成功したことを報告した。今回は *B. plicatilis* が大増殖をし，その捕食作用により micronalgae が急減し，水が透明になるにつれて池水の pH 並に溶存酸素がどのように変化するか。果して養鰻池における水変り時と同様に pH 並に O_2 が低下するかどうか。更に第1報に不充分であつた *B. plicatilis* 自身の個体数密度の変化を明かにすることを目的としてこの実験を行つた。

実　験　方　法

植物性プランクトンは，第1報と同様，津市島崎町黒宮養鰻池（第5号池）の *Synechococcus* sp. を用いた（昭和 30 年 6 月 28 日採水）。池水 20 L を実験室に持ちかえり，前報同様にプランクトン・ネットで動物性プランクトンその他を取除きその濾液を用いた（*Synechococcus* は極めて小さく，大きさ 1～2μ でネットの網目を通過する)。*Synechococcus* sp. を多量に含み（池水 1 cc について 13.29×10^6 細胞）鮮緑色を呈する濾液を今回は大型ガラス角水槽（26.5×21×30 cm）に 8 L を入れ，それに KNO_3 溶液を 17.5 mg/L（N として），KH_2PO_4 溶液を 2.50 mg/L（P_2O_5 として）になるように加え 1 日放置して後実験に用いた。

輪虫類，*Brachionus plicatilis* は第1報と同じく津市栗真町屋町加藤養魚場養鰻池（第2号池）から 6 月 28 日に採集したものを用いた。この池は第二次の水変り中であり，プランクトンは殆んど輪虫類から成り，その中で *B. plicatilis* が殆んど大部分を占めていた。この輪虫をネットで水変り池水中に濃縮して持ちかえり実験に供した。

前記の 8 L の *Synechococcus* 池水に，その 1 cc について 30 個体の割合（輪虫池水 200 cc に相当する）に *Brachionus plicatilis* を加え，1 週間に亘り，水色，透明度，*Synechococcus* の細胞数，*B. plicatilis* の個体数密度，pH，溶存酸素等の変化を追跡した。これと同型同容の水槽に上記と全く同様の処置を行い，唯 *B. plicatilis* を加えないものを control とし，両水槽を直射日光のあたらない窓側の机上に並列において比較に供した。

Synechococcus の細胞数の計数方法は第1報と全く同じである。

B. plicatilis の個体数の変化は，水槽中で水をよく攪拌して均一に分布させるようにし，サイフォンを用いて中層から 40 cc をとり，それにフォルマリンを加えて遠心分離器にかけて沈澱させ，2 cc に濃縮し，その 1/4（0.5 cc）を counting cell に入れて全個体数を計数し，池水 1 cc あたりの個体数を算出した。

溶存酸素はサイフォンにより略々中層から酸素瓶に採水し，Winkler 法によつて定量し，1 部ガス分析法（菅原，1941）を併用した。ガス分析は本学水産学部岩井寿夫氏の協力によつた。pH は比色法により P.R., T.B. を用いた。

この実験は昭和 30 年 6 月 29 日から 7 月 6 日に亘つて行い，その間水温は 26.8°C ～31.1°C であつた。

Table 1. The changes of some water conditions and micron-algae in number, caused by the abnormal reproduction of *Brachionus plicatilis*

		Before	After 1 day	2 days	3 days	4 days	5 days	6 days	7 days
	Date	29. VI	30. VI	1. VII	2. VII	3. VII	4. VII	5. VII	6. VII
	Time	15.30	15.30	9.30	17.00	17.00	16.00	16.00	9.10
	Weather	fine	fine	cloudy	fine	fine	cloudy	rainy	cloudy
Addition of *B. plicatilis*, 30 inds. per 1 cc	Water-color	green	brownish green	yellowish brown	yellowish brown	clear	clear	clear	clear
	W.T. °C	27.8	28.1	26.5	30.2	29.5	29.2	27.9	26.8
	pH	8.3	7.3	7.5	7.8	6.9	6.9	7.0	7.3
	O_2 cc/L	5.07	3.01	2.85	3.73	4.41	11.30*)	12.38*)	11.96*) (4.36)**)
	O_2 %	91.2	54.5	50.0	70.1	81.8	208.5*)	223.1*)	211.3*) (77.1)**)
	Cell number of micron-algae /cc	13.38×10^6	5.44	0.199	0.066	0.055	0.063	0.070	0.063
	Rotifer /cc	30	47	77	102	76	60	4	0.7
Control	Water-color	green	〃	〃	〃	〃	〃	brownish green	yellowish brown
	W.T. °C	27.8	28.1	26.5	31.1	29.9	29.2	27.9	26.8
	pH	8.3	8.3	7.8	9.0	9.0	8.8	8.3	7.3
	O_2 cc/L	4.97	5.15	4.13	12.92	11.60	10.88	3.67	3.82 (4.01)**)
	O_2 %	89.4	93.2	72.5	247.0	216.8	200.7	66.1	67.5 (70.9)**)
	Cell number of micron-algae /cc	13.19×10^6	13.81	15.19	12.44	11.75	12.63	3.63	0.121
	Rotifer /cc	—	—	—	—	—	—	6***)	13***)

*), By Winkler's iodometry. **), By Sugawara's carbon dioxyde method. ***), *Brachionus urceolaris*.

実 験 結 果

1. 水色.—水色並に透明度の変化は第1報の結果より更に顕著で，鮮緑色を呈した池水は，*B. plicatilis* を加えて1日後には黄色を帯びた褐緑色に変り同時に著しく澄んで，2日後には黄褐色に澄み，*B. plicatilis* の各個体を肉眼で透視出来る位に透明になり，黄褐色は3日後まで続き，4日後から殆んど無色透明になつた。これに対し輪虫を加えないcontrol は 5 日後までは鮮緑色がそのまま維持され，輪虫を加えたものと著しい対照をなしていた。しかし 6 日後には奇妙な現象がおこり，黄緑褐色となり稍々澄みはじめ，7 日後には著しく透明になり，水色は黄褐色に変つた。control の 6 日後における水色並に透明度の変化の原因については後述する。

全体的に見ると，今回の実験における水色並に透明度変化の様式並に速度は第 1 報の No. 5 (R. 3200) に略々一致する。

2. 植物性プランクトン，*Synechococcus* sp.—*Brachionus plicatilis* の捕食作用による micron-algae, *Synechococcus* sp. の減少傾向は全体的に見ると，第1報の No. 5 に類似するが，それより稍々顕著である。即ち *Synechococcus* の細胞数は1日後には最初の約 1/2.5 に，2 日後には 1/67 に，3 日後には 1/203 に，4 日後に最少となり，0.055×10^6 に減少し，最初の 1/243 になり，その後 7 日後まで大差はない。従つて 1～3 日間の変化が最も大きい。

これに対し control では細胞数は5日間は $11\sim15\times10^6$ を維持し変化はない。しかし6 日後から急激に減少しはじめ，7 日後には 0.121×10^6 に減じ，*B. plicatilis* を加えたものと大差ない状態に減少した。この原因については後述する。

3. 輪虫類の個体数密度の変化.—前報では*Brachionus plicatilis* 自身の個体数密度の細かい変化は明かにすることが出来なかつたが，本実験の結果，短時日の間に極めて大きい変化の存在が明かになつた。(Table 1 and Fig. 1)。

加えられた *B. plicatilis* は 3 日後まで急激に増殖し，最初の個体数密度，30 個体/ccは，1 日後には約 1.5 倍，2 日後には 2.6 倍，3 日後には最大の個体数密度，102 個体/ccになり，最初の 3.4 倍に増加した。それから急激に減少しはじめ，4 日後には 76 個体/cc，6 日後には 4 個体/cc となり，最初に加えた個体数密度より却つて小さくなり，その 1/7.5 に減少し，7 日後には殆んど斃死し，0.7 個体/cc になつた。この輪虫の顕著な増殖力と最大個体数密度に達して後の急激な斃死は養鰻池の水変りの経過を考察する上に注目すべき重要な事柄であろう。

control では最初の間には輪虫類並に他のプランクトン動物は殆んど認められなかつたが，前述したように 6 日後から水色が変化し透明になりはじめ，*Synechococcus* の細胞数も急激に減少したので，検鏡しよく調べたところ，輪虫類の *Brachionus ureceolaris* 並に原虫類の *Euplotes* sp. (Ciliata) が多数増殖し，個体数密度は6日後には *B. urceolaris* が 1 cc について 6 個体，*Euplotes* sp. が 1 cc について 110 個体，7 日後には *B. urceolaris* が 13 個体/cc，*Euplotes* が 14 個体/cc であつた。検鏡結果によると，両種の体内はいづれも著しい緑色を呈していたので，control における 6 日後の *Synechococcus* の急激な減少は，この control 内で増殖した両種の捕食作用に依存するのではないかと思われる。

4. **pH.**—実験前は両水槽共に pH は 8.3 を示していたが，*B. plicatilis* を加えたものは，1 日後には急激に 7.3 に低下し，3 日後には明るい窓側に寄せたので，植物性プランクトンの炭酸同化作用の増進により pH は 7.8 に上昇したが，4〜5 日後には 6.9 に低下し，6〜7 日後には 7.0〜7.3 程度になつた。これに対し control においては pH は著しく高く，晴天には 9.0，曇天並に雨天には 7.8〜8.3 を示し，輪虫を加えたものと著しい対照をなした。(Fig. 1)。しかし 7 日後には植物性プランクトンの減少並に *B. urceolaris*, *Euplotes* sp. の著しい増殖のためか pH は 7.3 に低下し，両者の差は失われた。

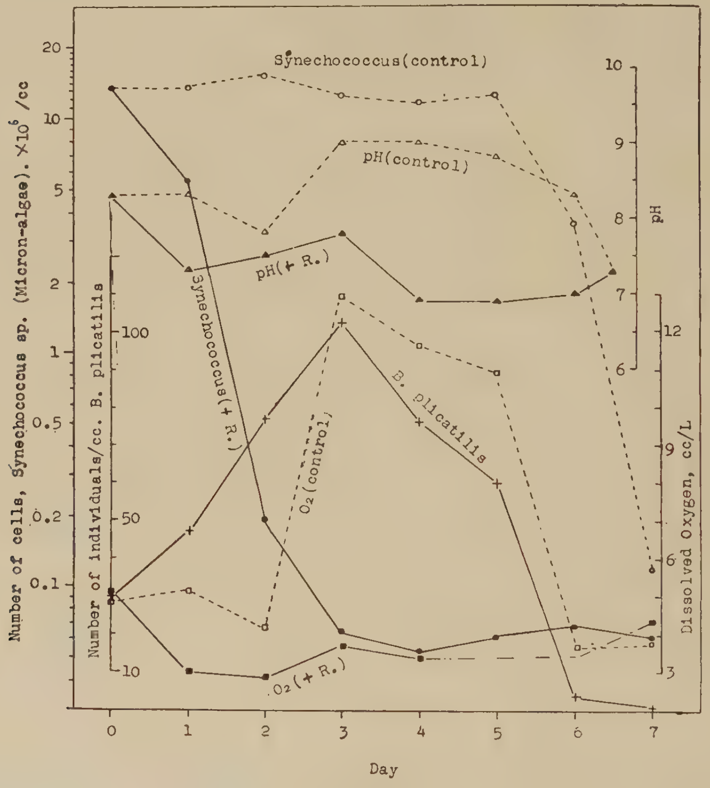

Fig. 1. The changes of some water conditions and *Synechococcus* (Micron-algae), caused by the abnormal reproduction of *Brachionus plicatilis*.

5. **溶存酸素.**—池水の溶存酸素量は水の pH と相関し，*B. plicatilis* を加えたものは，1 日後から急激に減少し，晴天の場合でも 50〜82% で飽和量に達しない。しかし 5 日後から奇妙な現象が見られ，Winkler 法のチオ硫酸ソーダによる滴定の折に終点が容易に定まらず，従つて溶存酸素量は過大になつた (Table 1)。この O_2 量は pH と相関しないので不合理である。7 日後にガス分析法によつて定量した結果は溶存酸素は 4.36 cc/L, 77.1% であつた。これ位の値が略々妥当のように思われる。この奇妙な現象は 4 日後から僅かに認められたが、それ以前及び control については見られなかつた。これは *B. plicatilis* の急激な斃死後にあたつているので，恐らく大量の斃死体のの分解の影響では

ないかと思われる。

control においては晴天には 10.88～12.92 cc/L（201～247%）に達し，その他のものも常に輪虫を加えたものに比べて溶存酸素量は著しく大きい。しかし *Synechococcus* の急減した 6～7 日後には溶存酸素は減少し，3.67～3.82 cc/L (66～68%) になり，輪虫を加えたものと略々同程度になつた。

本研究の結果を要約すると，輪虫類，*Brachionus plicatilis* の大増殖とその顕著な捕食作用によつて植物性 nannoplankton，*Synechococcus* sp. は急激に 1～3 日の間に殆んど消滅し，それに伴い水色は緑色から黄褐緑色，黄褐色を経て無色透明になり，水の pH は 6.9～7.3 位に低下し，溶存酸素は 3～4 cc/L (50～70%) 位に減少する。この実験室内の現象は自然の養鰻池における水変り現象に類似する。

考　　察

上述の実験室内における *Brachionus plicatilis* の異常増殖とその著しい捕食作用による micron-algae，*Synechococcus* の急激な消滅に由来する“水変り”類似の現象を天然の養鰻池における“水変り”と対比して見る。

Table 2 並に Table 3 は夫々伊藤・岩井（未発表）の三重県養鰻池の調査資料並に稲葉 (1937) により静岡県浜名地方の養鰻池について調査された報告の中から“水変り”中並に後の例を摘記したものである。

Table 2 並に Table 3 から，三重並に静岡の養鰻池における水変りに関し，次のような共通的特性を引き出すことが出来る。第 1 には水変りには殆んどすべての場合，輪虫類の Brachionidae に属する *Brachionus calyciflorus*, *B. ureceolaris*, *B. plicatilis* の大増殖が見られる。三重の No. 01, No. 04, No. 003 池では橈脚類，*Pseudodiaptomus inopinus*, *Sinocalanus tenellus* が優占群であり，輪虫類の大増殖は見られない。又静岡の No. 6 池には見るべきプランクトンはない。これらは水変りの最盛期を過ぎ，上述のように輪虫類が over population の影響で斃死し，既に回復の時期に観測がなされたものと思われる。第 2 に植物性プランクトンは輪虫類の大増殖とは全く逆に消滅するか，或いは著しく減少し，水色は緑色から暗緑色，黄褐色，或いは乳白色に変り，更に無色透明に近くなり，透明度は著しく大きくなる。しかし *Microcystis*, *Anabaena*, *Scenedesmus* 等の大型のプランクトン植物は水変り時の輪虫類の大増殖の池水中に共存する点は興味深く重要な事柄であろう。これは輪虫類の Brachionidae は所謂 nannoplankton に属する micron-algae を主な餌料とし，上述の 3 種等の大型のものは捕食が不可能か或いは餌料として不適当であるためと考えられるが，輪虫類の捕食作用と被捕食者としてのプランクトン植物の種類と大きさとの関係はこれからの研究課題である。

第 3 に水変り池水の pH は著しく低下し，普通は pH＝6.9～7.4 程度である。また溶存酸素も著しく減少し 0.8～4.2 cc/L 程度になる。

第 4 に水変りの生起は急激であり，一般に 1～2 日の間に急変する。

上述の自然の養鰻池における水変りの特異性と本実験における輪虫類，*Brachionus plicatilis* の異常増殖とその micron-algae の顕著な捕食作用による水変り類似の現象は，水色，透明度の変化速度，植物性プランクトンの減少率，及び池水の pH の点で極めて

Table 2. The plankton and water conditions of "Mizukawari" eel-culture ponds in Mie Prefecture.

(ITO and IWAI)

Pond number	Position	Date	W.T. (°C)	O_2		pH	Cl g/L	Water color	Dominant plankters
				cc/L	%				
208	Kumozu	9. IX. '53	22.9	3.29	53.2	7.3	3.268	yellowish brown	*Brachionus plicatilis* cccc, *Pseudodiaptomus inopinus* cc.
408	Tsu	19. IX. '53	25.8	1.58	27.4	7.7	5.828	brown	*B. plicatilis* ccc, *B. urceolaris* cc, Phytoplankton ±
613	Edobashi	31. VIII. '54	27.8	3.70	66.0	7.3	4.592	brown	*B. plicatilis* cccc.
616	Edobashi	31. VIII. '54	28.5	4.00	73.3	7.2	1.896	yellowish brown	*B. plicatilis* ccc, *Pseudodia. inopinus* cc.
703	Shiroko	12. X. '54	18.3	4.20	62.5	6.9	0.440	milky yellowish green	*Brachionus calyciflorus* ccc, *Microcystis* ±
1011	Kawagoe	19. X. '54	19.0	4.20	64.6	7.1	1.096	brown	*B. calyciflorus* ccc, *Microcystis* ±
01	Matsugasaki	21. X. '54	19.3	2.80	43.1	6.9	2.192	clear	*Sinocalanus tenellus* ccc.
04	Matsugasaki	21. X. '54	19.2	2.20	34.5	6.9	1.024	milky green	*S. tenellus* ccc.
003	Matsugasaki	22. X. '54	18.6	3.30	49.4	7.1	2.752	milky brown	*S. tenellus* ccc, *P. inopinus* ccc.

Table 3. The Plankton and some environmental conditions of "Mizukawari" eel-culture ponds around Lake Hamana, Shizuoka Prefecture.

INABA (1937)

Pond number	Date	W.T. (°C)	O_2		pH	Cl g/L	Water color	Dominant plankters
			cc/L	%				
1	26. V. '34	23.0	1.08	17.7	6.4	—	clear	*Brachionus urceolaris* ccc, *Microcystis* ±
2	6. V. '34	20.7	2.05	32.3	7.4	—	clear	Brachionidae ccc.
3	9. V. '34	21.6	0.76	12.1	7.4	—	〃	〃
4	5. V. '34	20.5	1.89	29.7	7.2	—	〃	〃
5	11. V. '34	20.5	1.38	21.6	7.4	—	〃	〃
6	23. V. '35	21.3	0.84	13.4	—	—	clear	No plankton
7	14. V. '34	—	—	—	7.4	2.86	clear	*B. urceolaris* ccc, *Microcystis* ±
8	4. V. '34	—	—	—	7.0	3.27	clear	*B. urceolaris* ccc, *B. caryciflorus* c, *Scenedesmus* cc.
9	23. V. '35	—	—	—	7.2	1.64	greenish brown	*B. urceolaris* cc, *Scenedesmus* c.
10	5. V. '35	—	—	—	7.2	2.91	brown	*B. urceolaris* cc, *Oscillatoria* c.
11	9. VII. '35	—	—	—	8.2	0.44	milky green	*B. calyciflorus* cc, *Microcystis* c.
12	12. VII. '35	—	—	—	7.3	0.75	dark green	*B. calyciflorus* +, *Scenedesmus* c.
13	25. V. '36	—	—	—	6.8	0.42	clear	*B. urceolaris* ccc, *B. calyciflorus* c.

よく一致する。ただ溶存酸素量は本実験の結果は 3～4 cc/L (50～70%) で養鰻池の水変り時より稍々多い。しかし実際の養鰻池にはウナギその他の養殖魚，底泥及び魚族の排泄物，餌の残部等が存在し，それらの呼吸作用並に分解作用による溶存酸素の消費も甚だしく，本研究における実験値以上に溶存酸素は少くなるであろうことは容易に考え得ることである。

稲葉峻 (1937) は養鰻池における水変りの原因と現象を，栄養塩類の欠乏によつて植物性プランクトンが枯死し，池水は透明になり，pH は低下し，溶存酸素は減少し，このような異常環境に適応した Brachionidae の輪虫類が大増殖をし，その呼吸作用によつて pH 並に O_2 は益々低下する。輪虫類は酸素欠乏水域を好み，しかも水変りによつて生じた豊富な餌料によつて大増殖をするとのべ，従つて輪虫類の異常増殖は水変りの原因ではなくてその結果と解釈した。しかし本実験の結果並に第 1 報によると，N, P 等の栄養塩類は充分に与え，しかも輪虫類を加えない control では植物性プランクトンは却つて増加

するのにも拘らず，輪虫類を投入したものはその個体数密度に比例した速度で植物性プランクトンが減少し，養鰻池におけると同様の水変りがおこることからみて，少くとも輪虫類の異常増殖を伴つた水変りにおいては，輪虫類の異常増殖は水変りの結果ではなくて，その大増殖とその顕著な植物性 nannoplankton の捕食作用が水変りの原因であると考えるのが妥当であろう。

天然の淡水湖においても，植物性 nannoplankton と輪虫類との消長には極めて密接な関係があり，被捕食者としての植物性 nannoplankton の消長は輪虫類の消長に依存していることが知られている。(Coldite, F. V., 1914, Z. f. Wiss. Zool., 108, 4, 520～630; Lantzsch, K., 1914, Z. f. Wiss. Zool., 108, 4, 631; Diefenbach, H. and R. Sachse, 1911, Int. Rev., Biol. Suppl. 3, 1; 上記文献は上野，1935 より参照)。

摘 要

1) 植物性プランクトン，micron-algae に属する *Synechococcus* sp. が著しく繁殖し (13.38×10^6 細胞/cc)，鮮緑色を呈する養鰻池水に水変り池の輪虫類，*Brachionus plicatilis* を 30 個体/cc の割合に加えると，*Synechococcus* sp. は輪虫類の顕著な捕食作用により，1 日後から急激に減少しはじめ，3 日後には *Synechococcus* は 0.066×10^6 細胞/cc になり，実験前の約 1/200 に減少し，水色は無色透明になる。

2) 輪虫類の *Brachionus plicatilis* は注加後 3 日後までに急激に増殖し，最大 102 個体/cc となる。しかし 4 日後から逆に急激に斃死しはじめ，7 日後には殆んど消滅し，0.7 個体/cc 程度になる。これはその over population による食物欠乏並に環境条件の変化によると思われる。

3) 池水の pH は 6.9～7.3 に低下し，溶存酸素も著しく減少し 3～4 cc/L (50～70%) 程度になる。

4) 静岡及び三重県の養鰻池における水変り現象の 22 例を挙げ，水色，透明度，植物性プランクトン，動物性プランクトン，pH 並に溶存酸素の点から“水変り”の特異性を帰納し，それを本実験の結果と比較し，本実験における *B. plicatilis* の異常増殖とその micron-algae の捕食作用による“水変り”類似の現象は自然の養鰻池における輪虫類の大増殖を伴つた“水変り”の現象と全く一致することを明かにした。

5) 従つて養鰻池の水変り時における輪虫類の異常増殖は，栄養塩類欠乏による植物性プランクトンの枯死に由来する水変りの結果ではなくて，水変りの原因か或いはその 1 部であるといえよう。

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